XIII. *The Effect of Environment on the Development of Echinoderm Larvæ: an Experimental Inquiry into the Causes of Variation.*

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Contents.

Introductory.

The influence of external conditions upon the growth and development of plants and animals has been known for a very long time, and has been made the subject of careful observation by many horticulturists and breeders, in addition to those who have attacked the problem in a more scientific spirit. As a rule, the changed conditions of environment, of which the effects were to be observed, were very considerable, and not such as might occur in nature, and the effects produced were not generally subjected to exact measurement. It seemed of interest, therefore, to determine as exactly as possible by measurement the effects which such slight changes in environment, as might occur under natural conditions, would produce

in the growth of some organism, with a view to ascertaining how far the variations in size and relation of parts which occur in all animals may be caused by these external conditions, apart from such variations which arise from intrinsic differences in the germ. The animal chosen for this purpose was the larva or pluteus of the sea-urchin, *Strongylocentrotus lividus*. These larvæ have the merit of being very hardy, and they develop readily from artificial fertilisations without any special precautions being taken. Moreover, it was found that these artificial fertilisations could be effected at all times of the year, irrespective of season. The chief objection to the choice of this animal lies in the fact that the growth of the larvæ cannot be carried to the adult stage, so that it is only possible to measure the effect of environment at a particular period in their development.

The plan of operations was very simple. About six or eight sea-urchins, which, as a rule, had been freshly obtained the same morning, were cut open, and pieces of the ovaries of each of the three or four female specimens shaken with forceps in a small jar of sea-water. Pieces of the testes were shaken in another jar, and the contents of the two jars mixed and stirred, the temperature being meanwhile noted. After standing an hour portions of this water containing the artificially fertilised ova were poured into glass jars containing 2 to $3\frac{1}{2}$ litres of sea-water. These jars were then transferred to a large glass tank, through which a stream of sea-water circulated, and were allowed to remain there throughout the whole period of development. Evaporation was prevented by covering the jars with glass lids. As a rule, the development was allowed to proceed for eight days, as the arms of the plutei attain their maximum development by the end of this time. A volume of saturated corrosive sublimate solution was then poured in each jar, such that the water should contain about ·25 per cent. of it. In a few minutes all the larvæ had sunk to the bottom. The supernatant liquid was poured off, and the larvæ with 100 or 200 cub. centims. of water transferred to a small beaker, from which more of the liquid was poured off. The larvæ were then washed in distilled water, and then in 50 per cent. alcohol. They were finally transferred to, and preserved in, 70 per cent. alcohol. To measure the larvæ, they were washed on the slide in water, and mounted in glycerine. Several hundred, in positions suitable for measurement, could be obtained on a single slide. The larvæ were measured under the microscope with a micrometer eyepiece, Zeiss, Obj. CC. Ocular, No. 3. The position of each larva measured was observed on the mechanical stage, and noted, so that it could not by mistake be measured twice. Three measurements were made, namely, the body length AB, the aboral arm length AC, and the oral arm length AD. The length of the calcareous skeleton in the body and limbs was always measured, in preference to the soft tissues surrounding it, as it is sharply defined, and therefore more suitable for the purpose. It is moreover of practically the same length as the soft tissues. In Fig. 1 are shown the measurements made. Only larvæ in the position indicated in this figure were measured, as with larvæ in the position indicated in Fig. 2, the oral arm is foreshortened.

It was a matter of difficulty to decide what number of larvæ should be measured in each case. Fifty was the number decided upon, as it was thought better to make several different fertilisations and developments under the particular conditions of environment to be examined, and measure 50 of the larvæ in each case, and to then take a mean of these several distinct sets of observations, than to carry out only one artificial development, and to measure a much larger number of larvæ. There are always slight variations in the environment which are unknown and not measurable, which produce as large variations in the determinations of the mean size of the larvæ, as are caused by measuring only 50 larvæ in preference to, say 200. The actual number of developing larvæ in a given volume of water was found to have practically no effect upon the body length, if it did not reach above about 30,000 per litre, but upon the arm length the effect is very considerable, as will be shown subsequently. It was therefore necessary to determine the number of larvæ present in each case. This was done by taking such a volume of the water as contained about 100 larvæ, adding corrosive sublimate, and transferring the larvæ to a small glass cell, in which they were counted under the microscope.

Fig. 1.

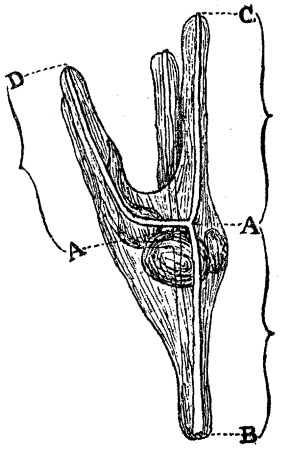
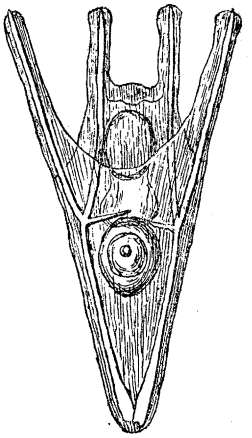


Fig. 2.



The details of the results obtained are given in the table at the end of the paper. In all, 10,000, larvæ were measured. The dates at which the fertilisations were performed are given, as the season was found to have a very considerable effect upon the size of the larvæ. The temperature of the water at the time of impregnation is given, and also the subsequent mean temperature during development. The temperature of the water was noted every day, generally at about the same time in the morning, and the mean of all the temperatures taken. As the tank in which the jars stood held a considerable volume of water, the temperature as a rule varied less than half a degree in the course of a day, and not more than a degree or a degree and a half during the whole course of the development. The time in days during which the larvæ were allowed to develop represents the exact time to within about

an hour, as both the fertilisations and the subsequent preservations of the larvæ were performed at about the same hour in the morning. The mean body length of the larvæ is given in arbitrary units, viz., those of the scale of the micrometer eyepiece. As the results are only comparative, it was not thought worth while to reduce these body lengths to fractions of a millimetre. Repeated measurements showed that on an average 152·3 units of the scale equal 1 millim. The lengths of the aboral and oral arms of each larva were reduced to percentages on the body length, and the means of the values determined. The figures in the remaining columns of the table will be discussed later on.

The Effect of Temperature on Development.

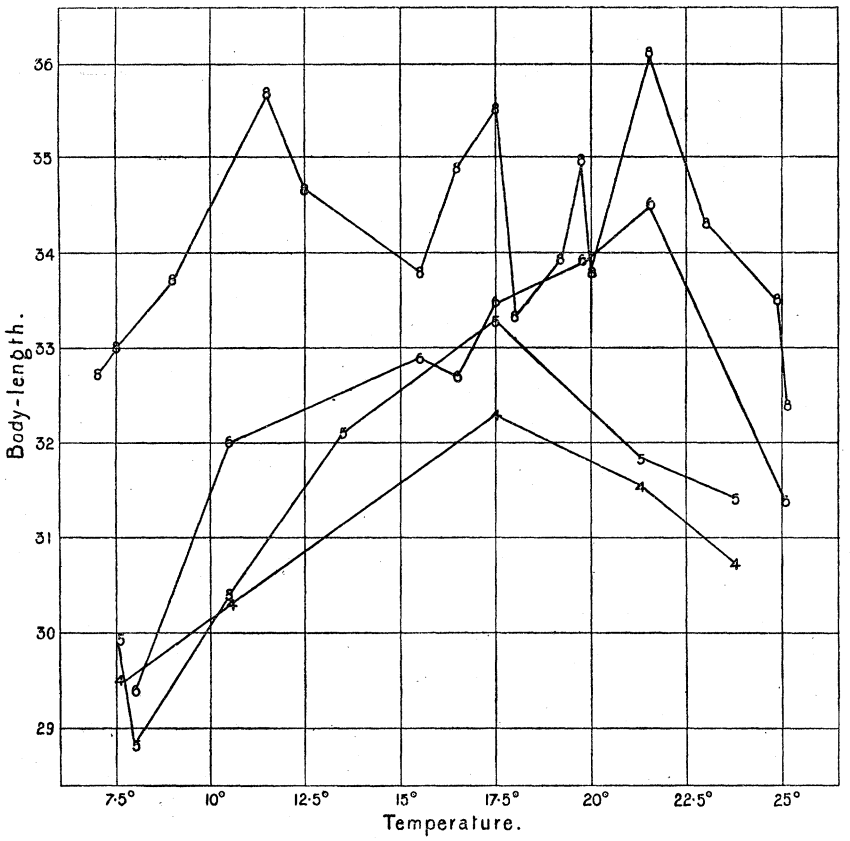
The effect of temperature on the development of animals is well known. As an instance, we may cite the striking experiments of HIGGINBOTTOM* on the development of the frog. Ova were allowed to develop, under otherwise similar conditions, at the temperatures 15°·6, 13°·3, 11°·7, and 10°·5 C. respectively. The tadpoles were observed to leave the ova 9, 14, 20, and 20 days respectively after the commencement of the experiments, whilst frogs were fully developed in 72, 160, 160, and 234 days respectively after the commencement. Thus a difference of 5° C. more than trebled the period of development.

In the present experiments the effect of temperature was studied at greater length than any other environmental condition. It was thought highly probable that variations in the temperature would have different effects or different degrees of effect at different stages in the development of the organism. The effect at the time of impregnation was first studied. The results obtained are somewhat remarkable. It was found that by keeping the water for an hour at a temperature of about 8° to 10°, or at about 25°, during the time of impregnation, the larvæ, which afterwards developed, were some 5 per cent. smaller in body length than those in which the ova had been impregnated at 17° to 20°, though the subsequent conditions of development had been identical. The effect of the difference of temperature for one hour during impregnation persists through the 191 hours of subsequent development. In Fig. 3 are shown the effects of temperature for one hour, during impregnation, upon the body length of larvæ after 4, 5, 6, and 8 days' development, the conditions of development being in all cases as nearly as possible identical. Only results of fertilisations performed before the beginning of June are embodied in this figure, as after this time the effects of season upon the size of the larvæ becomes so marked. The figures in the curves represent the number of days of development. As might be expected, the curves are very irregular, but they indicate clearly that the larvæ reach their maximum body length when impregnation is performed at about 17°·5 to 21°·5, whilst at temperatures above or below these limits, the larvæ become smaller, and that too in

* 'Phil. Trans.,' 1850, p. 431.

greater proportion the more the temperature varies from the favourable limits. These curves also show that the larvæ increase steadily in size as the period of development increases from four to eight days. They also seem to show that the effect of low or high temperature during impregnation is more marked when the larvæ have developed 4 or 5 days, than after 8 days. As the curves are so irregular, this conclusion can only be looked upon as probable, and not with any degree of certainty. Temperatures above 26° were found to be fatal to the success of the fertilisation.

Fig. 3.



In order to thoroughly prove the effect of temperature at the time of impregnation upon the size of the subsequently developed larvæ, the observations were repeated several times during several months of the year. As the temperature for one hour during impregnation has such a considerable effect, it was thought to be of interest to determine the result if this period of abnormal temperature be still further reduced. In Experiments 63 and 65 the time was reduced to three minutes; in Experiments 101, 104, 107, 144 and 186 to one minute, and in Experiments 100, 103, 106 and 187 to ten seconds. Observations were also made in each case on the effect of one hour's abnormal temperature during impregnation. In the performance of these experiments, beakers of water containing the ova and spermatozoa were brought to the required

temperature and mixed. Ten seconds after mixing, a third of the solution was poured into one jar of water at the ordinary temperature; a minute after, another third into another jar, whilst the remaining third was kept for an hour. In the table are shown the percentage amounts of decrease produced in the size of the larvæ, on those in which the impregnation was performed at a normal temperature of about 20°. In all cases the period of the development of the larvæ was eight days.

Numbers of Experiments.	Date.	Temperature of impregnation of—		Kept during impregnation for—		
		Normal.	Abnormal.	1 hour.	1 or 3 minutes.	10 seconds.
		°	°	per cent.	per cent.	per cent.
24	April 24	19·8	25·1	3·3		
43	May 9	21·6	7·5	8·5		
44	,,	21·6	11·5	1·8		
51	May 17	17·6	11·2	8·3		
62, 63	May 25	20·0	25·1	13·8	10·6	
64, 65	,,	20·0	8·9	0·0	0·3	
100, 101, 102	June 13	19·2	5·5	1·2	6·9	2·4
103, 104, 105	,,	19·2	9·4	2·5	3·6	2·7
106, 107, 108	,,	19·2	25·7	4·7	1·9	+ 0·2
143, 144	July 25	20·3	25·7	+ 1·0	2·7	
145	,,	20·3	7·5	8·7		
184	Sept. 14	20·0	25·7	5·1		
185, 186, 187	,,	20·0	8·3	2·5	2·4	1·9

In this table are included only those observations in which the temperature of impregnation was either below 12° or above 25°. In 13 observations in which the time of impregnation at the abnormal temperature was one hour, the mean decrease is 4·6 per cent., the decrease for the eight observations at about 8° being 4·2 per cent., and for the five at 25°, 5·2 per cent. In the seven experiments in which the time was a minute it is 4·1 per cent., or somewhat more than in the seven corresponding experiments at an hour, which have a mean value of 3·4 per cent. In the four observations at 10 seconds, the mean decrease is 1·7 per cent., as compared with 3·7 per cent. for those at an hour. It is evident, therefore, that it is quite as effectual to keep the ova for only a minute during impregnation at the abnormal temperature, as to keep them an hour. In a minute's time little more than the impregnation of the ovum would be accomplished. The processes connected with the fusion of the male and female pronuclei and the commencement of segmentation can scarcely have begun, and hence it must be the temperature of the ovum and spermatozoon solely at the time of impregnation which is of such considerable influence on the subsequently developing larva. This curious result may be accounted for by supposing that the vibratile energy with which the spermatozoon strikes and enters the ovum affects the subsequent processes of segmentation. As the effect produced when the period of impregnation is only 10 seconds is smaller than when it

is a minute or an hour, we must conclude that the time is insufficient for all the ova to become impregnated at the abnormal temperature.

In two different cases the ova were observed to reach the two-celled stage about an hour and a quarter after impregnation, and the four-celled stage about an hour and three-quarters to two hours after. No ova were observed to have begun segmenting 55 minutes after impregnation. Hence, it may be concluded that the temperature of the impregnated ovum during the processes of fertilisation and the commencement of segmentation does not in a measurable degree affect the subsequent development.

A but too obvious feature of the above table is the great irregularity of the values. The numbers in the column for one hour's impregnation vary from — 13·8 per cent. to + 1·0 per cent., and those in the next column from — 10·6 per cent. to — 1·9 per cent. This is scarcely to be wondered at, considering all the possible sources of error. The mean size of the larvæ determined from fifty measurements may vary by about 2 per cent., and as the values in the above table are the differences between the sizes at normal and abnormal temperatures of impregnation, the probable error arising therefrom is doubled. There is another great source of error. In most of the fertilisations, the ovaries of three different sea-urchins and the testes of three were used, so that the peculiarities of particular individuals might produce as small an effect as possible, and average larvæ be obtained, whereby comparisons of the various fertilisations might be rendered more valuable. Now, as will be seen shortly, different sea-urchins give rise to larvæ differing widely in size, and hence the average size of the larvæ in a fertilisation would vary, according as more or less of the ova or sperma of each individual sea-urchin were shaken into the water for fertilisation. It was, perhaps erroneously, thought best when performing fertilisations at abnormal temperatures, to bring the water to the required temperature, and then shake parts of the ovaries and testes in it in similar proportions to those shaken in the water at a normal temperature. The alternative plan would have been to shake the ovaries and testes in only one volume of water in each case, and then bring different portions of the solutions to the required temperatures before mixing. As in the method adopted it would not be possible to hit off similar proportions at all exactly, there must have been, without doubt, an additional error introduced. That this error produces a considerable effect is rendered obvious by comparing the values for one hour's impregnation among themselves, and also with those at the corresponding temperatures for one minute, when it does not come in. Thus, the mean difference is reduced from 4·4, the value in the former case, to 2·4.

A few determinations were made on the effect of temperature during the course of the development of the larvæ. As in the case of the former observations, temperatures between about 17° and 22° appear to be the most favourable. In several cases, when the temperature of the tank sea-water was at a maximum, some of the larvæ were allowed to develop in another tank, through which circulated a current of fresh water, which was several degrees lower in temperature. The

larvæ in Experiments 147 to 151, developed under various conditions at 23°·1, are on an average 3·9 per cent. smaller than those in Experiments 152 to 156, of which the temperature was 19°·9, and in Experiments 164 to 169, the larvæ developed at 23°·3 are 4·9 per cent. smaller than those in Experiments 170 to 175, developed at 20°·7. In these cases, however, the larvæ were subjected to other changes in environmental conditions besides that of temperature. Of individual observations, in which all the conditions but that of temperature were the same, the larvæ in Experiment 138, kept at 23°·7 during development, are 2·4 per cent. smaller than those in Experiment 139, which were kept at 19°·9. In Experiment 147, the larvæ kept at 23°·1 are 1·8 per cent. smaller than those kept at 19°·9. In Experiments 164 and 169, the larvæ kept at 23°·3 are respectively 1·7 per cent. and 1·4 per cent. smaller than those in Experiments 170 and 175, which were kept at 20°·7. The most marked effect was produced in Experiments 55 and 56, in which the larvæ were kept at about 15°·7, by means of a more rapid stream of fresh water through the tank. The larvæ in Experiment 55 are 4·4 per cent. smaller than those in Experiment 52, which were kept at 19°·2, whilst those in Experiment 56, where in addition the impregnation was performed at 15°·5, are 6·9 per cent. smaller. It is possible that the temperature of the water is not unfavourable till it reaches above 22°, for in Experiments 53 and 54, where the larvæ were kept at 22°·1 during development, the average decrease is only ·1 per cent. on the larvæ kept at 19°·2. The most striking point about these results is the comparatively small effect produced in the larvæ by the unfavourable temperatures, when we remember the considerable decrease produced by a minute's or even ten seconds' unfavourable temperature during impregnation.

A few observations were made in which the temperature of impregnation varied between 15°·7 and 23°·7, the limits of variation of temperature during development. In Experiments 41 and 42, where the impregnation was performed at 16°·4, the larvæ are respectively 9 per cent. and 8·2 per cent. smaller than those impregnated at 21°·6, and in Experiment 193, where it was performed at 16°·0, the larvæ are 3·3 per cent. smaller than the normal. In Experiment 56, which has just been cited, the larvæ impregnated at 15°·5 are 2·5 per cent. smaller. Of observations at higher temperatures, the larvæ in Experiment 49, impregnated at 23°·1, are 3·5 per cent. smaller than those impregnated at 17°·0, and in Experiment 199, those impregnated at 23°·8 are ·4 per cent. smaller than those at 19°·5. In Experiments 8 and 9, measured after four and five days' development respectively, the larvæ impregnated at 23°·8 are on an average 1·9 per cent. smaller than those at 21°·3. On the other hand, in Experiment 92, the larvæ from the impregnation at 22°·9 are apparently 3·1 per cent. larger than those at 19°·3. We may, therefore, conclude that, whilst temperatures between about 17° and 22° are the most favourable for development, yet a temperature a degree or two above or below these limits does not produce a very marked effect, indeed probably but little greater than if the ova had been kept at the same temperature for a single minute during impregnation.

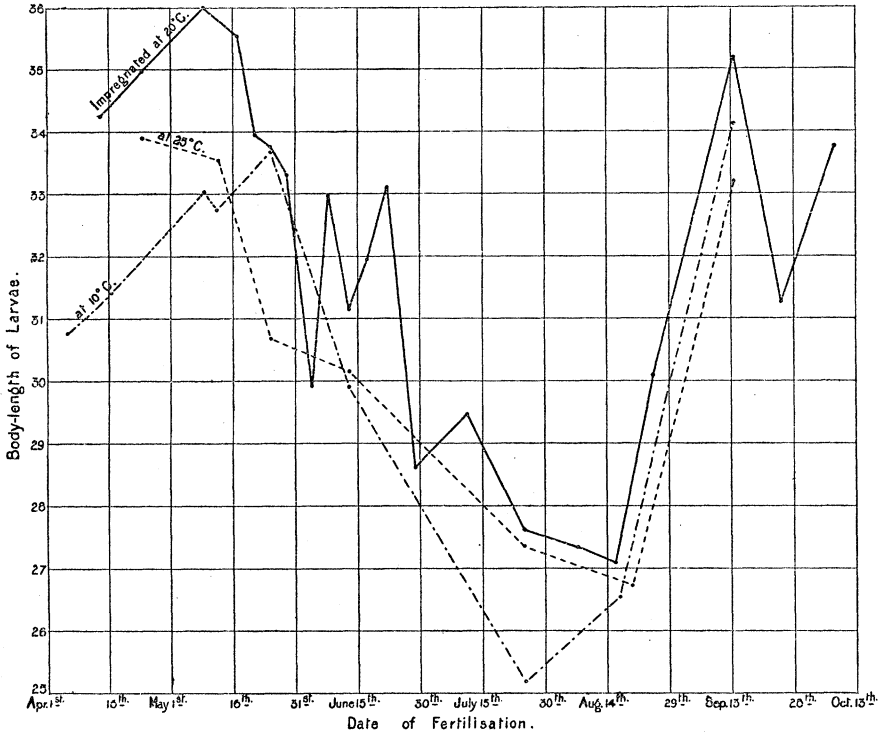
In Experiments 50 and 51 the fertilisations were kept at about 12° for ten hours subsequent to impregnation, after which they were kept at ordinary temperatures. The larvæ in Experiment 50 are 2·4 per cent. smaller than other larvæ kept at 23° for ten hours subsequent to impregnation (Experiment 48); whilst those in Experiment 51, which were kept in addition at 11°·2 during impregnation, are 10·5 per cent. smaller.

The effect of temperature upon the length of the arms of the larvæ will be discussed later on.

The Effect of Season on Development.

It has already been mentioned that season has a considerable effect upon the size of the larvæ. In Fig. 4 is shown graphically the relation of the size of the larvæ,

Fig. 4.



after eight days' normal development, to the time at which the fertilisation was performed. The mean body lengths of the larvæ where the impregnation was effected at about 8° to 10°, and at about 25°, are also indicated, by separate curves. Artificial fertilisations were carried out on an average nearly once a week, from the beginning of April to the beginning of October. The normal breeding season of *Strongylocentrotus lividus* is stated to be roughly from December to March.* These fertilisations, therefore, extend through the most unfavourable months of the year, during which little or no breeding occurs under natural conditions. They are, perhaps, the more

* Lo Bianco, 'Mittheilungen aus der Zoologischen Station zu Neapel,' VIII. Band, 3 Heft, 397.

interesting on that account, as they show how enormously the size of the larvæ is affected by the maturity of the ova and sperma. In the middle of August, which the curve shows to be the period of minimum maturity, the larvæ are 24·9 per cent. smaller than those from the fertilisation on May 9th, and about 20 per cent. smaller than the average size of the larvæ developed in April and May. About 2 per cent. of the diminished size is due to the higher temperature during development, but the immaturity of the ova and sperma is apparently the only cause to account for the remainder of the decrease in size of the larvæ. Thus, during these summer months the ovaries and testes were frequently much diminished in size, and yielded no ova or spermatozoa at all on shaking in water. Even the least affected ovaries seemed to contain a much smaller proportion of fertilisable ova than in the normal breeding season. The curve shows very distinctly the variability in the maturity of the individual sea-urchins. Thus, the larvæ of the fertilisation on June 4th are 10·3 per cent. smaller than those of May 29th; those of June 29th are 16·5 per cent. smaller than those of June 22nd; and those of August 25th are 14 per cent. smaller than those of September 14th. With regard to the comparative maturity at various months of the year, the curve shows it to be more or less constant during April and May, but to decrease rapidly through June and July, till it reaches a minimum about the middle of August. From this time it begins to increase very rapidly, much more rapidly, in fact, than it had decreased during June and July, till, by the beginning of October, it has reached the level it occupied in April and May.

As the effect of maturity is so marked in the sea-urchin, it does not seem improbable that it might have a certain amount of influence in higher animals, and that, for instance, the offspring of mammals, conceived just before the reproductive organs become functionless from age, should be, on an average, of smaller size than those conceived during their full maturity. This does not seem to be the case, however, as Mr. Galton has informed me, in a private communication, that a provisional inquiry made by him did not seem to show any difference between the children of old couples and those of ordinary couples, or any difference between the produce of old and young thoroughbred stallions.

In the curves of Fig. 4 the values given up till April 17th are only calculated ones. The larvæ in these observations were mostly measured after five or six days' development, and hence corrections obtained from other determinations have been made, to bring them up to their size after eight days' development. In those cases where two or more separate measurements were made at the same temperature, the mean value is given in the curves.

The Effect of the Salinity of the Sea-water.

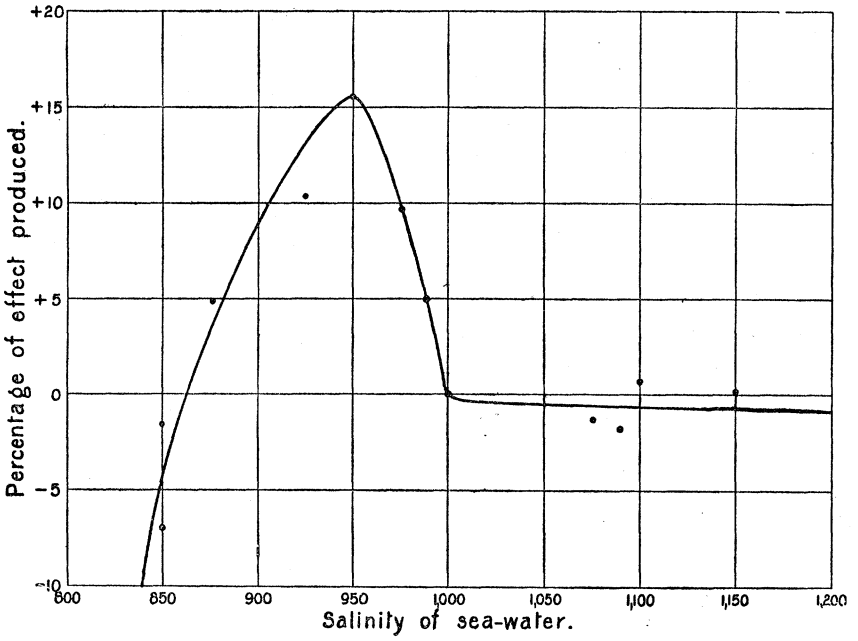
The effect of the salinity of the sea-water upon the growth of animals has been studied by Loeb.* He allowed long stems of the hydroid polyp *Tubularia mesem-*

* J. Loeb, 'Biological Lectures delivered at Woods Holl,' 1893, 46.

bryanthemum to grow in water of various degrees of salinity. He found that the maximum of growth took place, not in sea-water of normal concentration, but in water containing about two-thirds as much of the saline constituents. With water more diluted than this the growth began to decrease again, and with water more concentrated than the normal the growth was also less. LOEB accounts for these results by supposing that the growth of animals is determined by the same mechanical laws as with plants, and that, within certain limits, the more dilute the water the more diffuses into the cells of the Tubularians, and the more rapid their growth.

I have obtained somewhat similar results to these for the development of plutei. In Fig. 5 are shown the relative amounts of increase and decrease produced after eight

Fig. 5.



days' development in water of varying concentrations. On the abscissa are shown the volumes of sea-water of which the saline constituents are contained in 1 litre of the solution, and on the ordinate the percentage of change of size produced. It was not found possible to grow the larvæ in water of greater dilution than 850 cub. centims. diluted to a litre, or of greater concentration than with 1150 cub. centims. concentrated to a litre, but these limits are sufficient to indicate the relation of growth to salinity. It will be seen that the larvæ reach their maximum growth in a solution containing 50 cub. centims. of distilled water per litre, where they are 15·6 per cent. larger than those developed in water of normal concentration. With greater dilution than this they steadily decrease in size again, till in a solution containing 150 cub. centims. of distilled water per litre, they are on an average 4·3 per cent. *smaller* than the normal larvæ. It is remarkable what a very

pronounced effect is produced by such slight dilution of the water. When the solution contains only 25 cub. centims. of distilled water per litre, the larvæ are 9·5 per cent. larger than the normal, and when only 12·5 cub. centims., 5 per cent. larger. We see that the maximum of growth takes place at a very much slighter dilution than LOEB observed in Tubularians, where it occurred in solutions containing about 300 cub. centims. of distilled water per litre. The relation of growth to dilution no doubt varies considerably with different animals. LOEB even found that the eggs of *Fundulus* developed in a completely normal manner in absolutely fresh water.*

The curve shows that in more concentrated water the growth of the larvæ has, if anything, slightly decreased, but the amount is so small as to be scarcely capable of measurement. This result differs from LOEB'S conclusions concerning Tubularians, but he worked with solutions of greater concentration, and, moreover, he prepared his concentrated solutions by the addition of sodium chloride to ordinary sea-water. Now only about 3·1 per cent. out of the 3·5 per cent. of saline constituents contained in sea-water consists of sodium chloride, and hence the normal proportion of the salts present was altered, whereby an injurious effect might be produced. In the present experiments, the method adopted in the preparation of, for instance, a solution containing 1150 cub. centims. concentrated to a litre, was to evaporate 400 cub. centims. of sea-water down to 100 cub. centims., and then, on cooling, mix it with 1900 cub. centims. of normal sea-water.

In all the above experiments, the ova were kept for one hour in sea-water of normal salinity during impregnation. Experiments were also made in which the impregnation was performed in water of various degrees of salinity, whilst the development was allowed to take place in normal water. In the table are shown the results obtained.

Number of experiment.	Salinity of sea-water during impregnation.	Variation in size produced.	Correction due to dilution of water of development.	Absolute effect produced.
	cub. centim.	per cent.	per cent.	per cent.
182	750	− 1·8	− 2·5	−4·3
134	800	+16·1	−12·0	+4·1
174	850	+ 6·0	− 7·8	−1·8
156	875	+ 2·1	− 5·0	−2·9
194	950	− ·4	− 2·0	−2·4
169	1075	+ 3·2	..	+3·2
196	1100	− 3·4	..	−3·4
163	1115	+ 5·8	..	+5·8
175	1150	+ 3·0	..	+3·0
200	1150	+ 1·8	..	+1·8
195	1250	− 2·8	..	−2·8

* 'Archiv für die ges. Physiologie.' Vol. 55, p. 535.

As a slight dilution of the water in which larvæ are developing has such a marked effect, it was necessary to note the volume of the diluted water in which the impregnation was performed which was poured in each jar, and calculate and allow for the increase in the size of the larvæ thereby produced. The variation in the size of the larvæ due to the effects of impregnation alone are given in the last column of the table. As the values are not very consistent, it is not possible to come to very definite conclusions as to the effects produced, but it will be seen that, as a rule, impregnation in diluted water exerts a harmful effect, whilst impregnation in concentrated water exerts a beneficial effect upon the subsequent development. The mean decrease produced by the diluted water is 1·5 per cent., and the mean increase by the concentrated water, 1·6 per cent. As the corrections due to the dilution of the water of development are so large, it is possible that the apparent effect produced by impregnation in diluted water is only due to experimental error, and does not exist in fact. In two out of the six observations with concentrated water, a decrease in the size of the larvæ was produced, but as both of them were made in the same fertilisation, viz., that on September 25th, they have not much more weight than a single observation. It may thus be looked upon as highly probable that a favourable effect upon the subsequent growth of the larvæ is produced by impregnation in concentrated water. This seems a curious result when it is remembered that concentrated water acting during the whole period of development produces no effect at all, or else a slightly harmful one.

As slight changes in the salinity of the water produce such considerable effects upon the growth of the larvæ, it may be of interest to examine to what extent the specific gravity of sea-water varies at different times and places in nature. The specific gravity of the water used in these observations was determined several times, and found to be on an average 1·0271 at 15°·56 C. From the determinations by DITTMAR of the saline constituents of waters of various specific gravities,* it was calculated that a litre of water of the specific gravity 1·0250 would only contain as much salts as 900 cub. centims. of a water of specific gravity 1·0280. In the *Challenger* Expedition no observations of the specific gravity of Mediterranean waters were made, but in the North Atlantic, for instance, between Bermuda and New York, the specific gravity of the water varied from 1·02504 to 1·02723 at 15°·56 C.† All the waters examined in the expedition, with the exception of those obviously diluted with river water, varied from 1·02403 to 1·02778. Now we have seen that in a solution containing 50 cub. centims. of distilled water per litre, the larvæ increased 9·5 per cent. in size, and in a solution containing 100 cub. centims., 15·6 per cent. Between Bermuda and New York, therefore, the water varied in specific gravity enough to cause a difference of 13 per cent. in the size of larvæ developed under the extreme conditions. We cannot doubt, therefore, that the variations in

* '*Challenger* Expedition,' vol. 1, 78.

† BUCHANAN, *ibid.*, 13.

the specific gravity of sea water, which are of continual occurrence under normal conditions, must have a very considerable effect upon the size of the plutei larvæ developing in it.

The Effect of Light on Development.

The effect of light upon the development of animals, and especially that of the various colours of the spectrum, has been frequently studied. BECLARD* allowed the eggs of *Musca carnaria* to develop in glass vessels of various colours. After four or five days the larvæ showed considerable differences in size. The development was affected by the various colours in the following order: violet, blue, red, yellow, white, green; the larvæ in the violet light having developed to three times the size of those in the green. JUNG† allowed the ova of *Rana temporaria* to develop for four months in glass vessels which were placed in slightly larger vessels, the space between being filled with various coloured solutions. He found the development to be affected by the colours in the following order: violet, blue, yellow and white, red, green. JUNG found that, contrary to the conclusions of HIGGINBOTTOM,‡ who performed several careful series of experiments, the duration of the development is longer in darkness than in light. JUNG also found the development of the embryos of *Sepia officinalis* to be affected in a somewhat similar manner by light,§ the order being violet, blue, yellow and red, green. Lastly, FATIGATI,‖ working on Infusorians, found that violet light accelerated their development whilst green light retarded it. We see that in all the observations cited, green light was found to have the most retarding effect upon the development. The only exception to this rule was experienced by BECLARD, who found that in adult frogs the respiration was considerably greater in green light than in red.

Though the object of this research was to measure the effects of such variations in the environment as might occur under natural conditions, it was thought to be of interest to make a few observations upon the effects of the various colours of the spectrum upon development. The method adopted was similar to that made use of by JUNG. A 2-litre jar containing the developing larvæ was placed in a $3\frac{1}{2}$-litre jar, and the space between, about 12 millims. in width, filled with various coloured solutions. For the green colour, a concentrated solution of nickel nitrate was used, as recommended by JUNG. For the blue colour, in some cases, a saturated solution of copper sulphate; in others, an alcoholic solution of the aniline stain, Lyons blue, as recommended by JUNG. For the violet, an alcoholic solution of Parma violet. To obtain the red and yellow colours, it was thought to be simpler to paste pieces of the red and orange yellow papers which are used for photographic purposes on the outer

* 'Comptes Rendus,' XLVI., 441.

† 'Archives des Sciences Physiques et Naturelles,' 1880, IV., 573.

‡ *Ibid.*

§ 'Mittheilungen aus der Station zu Neapel,' II., 1880, 233.

‖ 'Comptes Rendus,' LXXXIX., 1st December, 1879.

surface of the inner jar. This paper lets through none of the rays of the spectrum beyond the yellow. Pieces of red and yellow paper were also placed on the mouths of these jars, so not a trace of white light could enter. In the case of the blue and green jars, this was not absolutely the case, for though a layer of coloured solution was formed between the bottoms of the two jars by allowing the inner jar to stand on an inverted glass dish, and though the mouth was covered up with a piece of black paper, yet there was about half-an-inch at the top of the jar exposed, where a small amount of white light could enter. Larvæ were also allowed to develop in absolute darkness, in a jar covered with black paper, and also in semi-darkness, in a jar with all but the bottom covered up. Here but a very slight amount of light could enter, as it would have to filter through the thick glass bottom of the tank and the glass bottoms of the two jars. The mouths of both these jars were covered with black paper. In the table are shown the percentage amounts of variation produced in the size of the larvæ after eight days' development under the various conditions.

Numbers of experiments.	Colour.	Percentage of change in size produced.	Mean change.
73, 140, 150	Semi-darkness	+4·3, +1·8, +1·4	+2·5
69, 91, 115, 161	Absolute darkness	−1·1, +1·0, −6·2, +1·0	−1·3
72, 114	Blue (copper sulphate)	−4·0, −4·9	−4·5
168, 181, 192. (116)	Green	+·3, −7·3, −7·3. (+·1)	−4·8
160, 167	Blue (Lyons blue)	−6·2, −8·6	−7·4
70, 112	Red	−5·8, −8·0	−6·9
71, 113	Yellow	−5·1, −12·8	−8·9

It will be seen that the effects produced are considerably different from those found by other observers for different animals. Almost every change from the normal white light brings about a decrease in the growth. The only exception is observed in the case of the larvæ grown in semi-darkness, where there is a slight but perceptible increase. The amount of decrease produced by growth in absolute darkness is so slight as to be almost within the limits of experimental error. Thus if Experiment 115 be excluded, it is nil. Blue light, when it is transmitted by copper sulphate solution, is less unfavourable than red and yellow light, as was found by other observers, but the blue light of Lyons blue solution, which was used by JUNG in his experiments, is just as unfavourable. The green light of nickel nitrate solution does not appear to be as unfavourable as red, yellow, or blue (Lyons blue solution), but the effect produced is probably more unfavourable than the figures show. Thus in two different cases, fertilised ova, which developed perfectly under normal conditions, were entirely killed off by the green light. The value in brackets under the heading of green light was obtained from larvæ grown in a green glass jar, where the green would be largely diluted with white light. It is unfortunate that the larvæ could not be successfully grown in violet light. Attempts were made five times, but in

every case after about the second day the solution went turbid and putrid, owing to the development of bacteria, the growth of which is favourably affected by the violet light. Water containing larvæ allowed to develop for the first two days in white light was affected in a similar manner. As far as can be gathered from the results obtained, even violet light has a deleterious effect upon the growth of the larvæ. Thus the larvæ developed in Lyons blue solution, which transmits a considerable proportion of violet rays, are much more unfavourably affected than those grown in copper sulphate solution, which transmits no violet rays, but a few red ones. The results obtained for developments in semi- and absolute darkness are useful as showing that the degree of opacity of the various coloured solutions and papers employed can have had but slight effect upon the development. Thus the red and yellow papers were much more opaque than the blue solutions, and these again more opaque than the green solution.

In all the above observations, the ova were kept for one hour during impregnation under normal conditions. As light and darkness had such slight effects when acting during the course of development, it was scarcely to be expected that they would produce a measurable effect when acting during impregnation. Such was found to be the case. In two experiments, Nos. 74 and 117, the ovaries and testes were shaken into water in the dull red light of a photographic dark room, and the solutions mixed and allowed to remain for an hour in absolute darkness. The mean variations in the size produced are — 1·1 and — ·5 per cent. In two other cases, Experiments 118 and 151, the solutions of ova and spermatozoa were mixed and allowed to stand an hour in bright sunlight, the jar containing the fertilisation being placed in a basin of cold water, to keep down the temperature. The variations produced are — 3·0 and + 1·2 per cent.

The Effect of Nutrition.

The effects which nutrition may have upon the development of animals have been frequently examined, and it is generally considered that this is one of the most powerful influences of environment in the production of variations. It will be unnecessary to refer at length to the experiments which have been made. A striking example is seen in the results obtained by JUNG,* for the development of tadpoles. After six weeks' growth, the individuals fed on fish or meat had developed to twice the size of those fed on aquatic plants, or on the coagulated albumen or yolk of fowl's eggs.

In the present research, but few observations were made upon the effects of nutrition, as there appeared to be enough food material present in the ova and in the water in which the larvæ were allowed to develop to satisfy all their wants. The water in which the impregnated ova were placed was taken from a large tank holding several thousand gallons, so that its composition was tolerably constant.

* 'Archives des Sciences Physiques et Naturelles,' tome VII., 225, 1882.

It contained hosts of organisms. In some cases, indeed, where comparatively large ones were present, they would eat up all the developing larvæ, and so had to be separately removed from the jars of water. If in the present observations the larvæ were in need of additional food, it is obvious that the larger the number of larvæ present in any given volume of water, the more would they be diminished in size. In the following table is given a list of all the observations made, where all the conditions of development were similar, but that of the number of larvæ present. On the one side of the table are given the mean size of the larvæ, where a smaller number of individuals per litre were present; on the other side, where a larger number.

Number of experiment.	Number of larvæ per litre.	Body-length.	Number of experiment.	Number of larvæ per litre.	Body-length.	Difference.
25	2600	33·18	28	7,440	32·65	− ·53
25	2600	33·18	32	18,650	32·86	− ·32
26	2600	33·24	29	7,440	34·39	+1·15
26	2600	33·24	33	18,650	33·53	+ ·29
26	2600	33·24	35	27,600	34·10	+ ·86
27	2600	33·33	30	7,400	34·17	+ ·84
31	7400	35·35	34	18,650	34·62	− ·73
63	2100	31·19	62	15,600	30·19	−1·00
65	8400	33·69	64	8,650	33·77	+ ·08
74	7900	32·98	67	15,550	33·34	+ ·36
93	700	32·33	87	5,400	32·93	+ ·60
101	1600	29·01	102	3,600	30·77	+1·76
104	1900	30·01	105	2,300	30·38	+ ·37
107	5000	30·55	108	9,700	29·68	− ·87
109	700	31·08	97	2,170	31·15	+ ·07
110	7200	31·95	111	11,800	31·10	− ·83
110	7200	31·95	117	20,000	31·38	− ·57
110	7200	31·95	118	12,600	30·60	−1·35
110	7200	31·95	119	10,800	30·55	−1·40
120	4100	33·07	125	9,100	34·07	+1·00
132	950	28·82	130	1,400	28·61	− ·21
135	160	29·45	136	900	29·79	+ ·34
135	160	29·45	137	510	29·69	+ ·24
143	700	27·88	144	900	26·85	−1·03
147	3500	27·34	151	6,100	27·68	+ ·34
156	1000	28 43	152	3,800	27·84	− ·59
170	1300	30·60	173	2,200	30·21	− ·39
185	7200	34·11	186	17,900	34·16	+ ·05
190	5900	30·32	189	7,900	31·39	+1·07
100	5900	30·32	188	11,100	31·24	+ ·92
Mean	3700		Mean	9,530		+ ·02

The conditions of observations made upon the effects of impregnation at various abnormal temperatures for a minute are looked upon as similar to those of the corresponding observations on impregnation for an hour. The conditions of larvæ allowed to develop in concentrated sea-water, in water containing small quantities of calcium

chloride, in stirred and not stirred water, and of larvæ from impregnations in absolute darkness and in sunlight, are also looked upon as similar to those of larvæ developed under normal conditions. The average number of larvæ in the one case is 3700 per litre, and in the other 9530 per litre. The mean body length is, on an average, ·02 greater for solutions containing the larger number of larvæ; hence it follows that the number of developing larvæ present in a volume of water has no measurable effect upon their size. There is one marked exception to this rule, which is not included in the table. In Experiment 36, there were present 39,000 larvæ per litre, and these larvæ are 4·8 per cent. smaller than the normal. As in Experiment 35, where 27,600 larvæ were present, there was no decrease in their size, we may conclude that it is only when more than about 30,000 per litre are developing together, a deleterious effect is brought about.

A few observations were made which may be considered to come under the heading of the effects of nutrition. In these the amount of spermatozoa added to the ova in impregnation was varied. In Experiment 37 about twenty times as much sperma was added as in the normal fertilisation, and in Experiment 60 about ten times as much. In these two cases the larvæ were respectively 8·0 and 12·8 per cent. smaller than the normal, but in each case the water went putrid about the second or third day of development, and remained so for two or three days. This fact is quite sufficient to account for the diminished size of the larvæ. On the other hand, in Experiment 90, where four times as much sperma was added, and in Experiments 158 and 177, where five times as much was added, and where there was no trace of putridity at any time during the course of the development, the larvæ vary respectively by + 6·0, + 5·1, and − 3·5 per cent., or on an average by + 2·5 per cent., from those developed under normal conditions. Within certain limits, therefore, the presence of additional sperma in the water containing the developing larvæ has probably a slightly beneficial effect upon their growth. The addition of only a very small quantity of sperma to the ova at the time of impregnation seems to have little or no harmful effect. In Experiments 59 and 177 only a tenth as much sperma was added as to the normal fertilisation. The larvæ were found to vary respectively by − ·6 and + ·5 per cent. from the normal.

It is of some little importance that the size of the larvæ should be comparatively so little affected by the proportion of sperma added, for it was of course impossible in the various series of fertilisations to maintain even an approximate relation between the amounts of ova and sperma. In the different fertilisations performed on the same day, it is probable that the amount of sperma added in any one case was not more than double that added in any other, as care was taken to try and keep the proportion as similar as possible.

The few other attempts made upon the effects of nutrition ended in failure. The addition of only 1 cub. centim. of egg albumin to 2 litres of water containing larvæ caused it to become putrid in a day or two, whilst the addition of a decigram of cane sugar resulted in fermentation.

The Effects of Products of Metabolism upon Development.

As will be seen subsequently, the actual number of larvæ developing in a given volume of water has a considerable influence upon the length of the oral and aboral arms, though it does not affect the body length. This was thought to be probably due to the influence of products of metabolism. To test this supposition, ova impregnated under normal conditions were allowed to develop in water in which larvæ had already been developing, these larvæ having been removed by filtration. In Experiment 57 the larvæ were developed in water in which 8000 per litre had previously developed for eight days. They are diminished in size by 7·9 per cent. In Experiments 76, 77, and 78 water was used in which 10,000 larvæ per litre had developed for ten days. The larvæ are diminished 7·3 per cent. in size in Experiment 76. In Experiment 77 the larvæ were kept in previous fertilisation water for nine hours immediately after impregnation, but there is no change in size. In Experiment 78 the ova were kept in ordinary water for nine hours after impregnation, when this water was poured off, and previous fertilisation water added. The larvæ have decreased 1·7 per cent. on those developed under normal conditions. It is evident, therefore, that certain of the products of metabolism exert a deleterious influence upon the growth of the larvæ. It may be wondered why in this case the size of the larvæ is not affected by the number actually present in the water. The probable reason is this. Two days after fertilisation the larvæ have developed to more than three-quarters of the size they attain after eight days development, and after three days to seven-eighths their size. By the second or third day, however, but a slight amount of metabolism has taken place, and even supposing that after the third day there were as much of the deleterious products present as in the water used in the above experiments, they would only diminish the size of the larvæ by 7 or 8 per cent. on the remaining eighth of body length they will put on between the third and eighth day; *i.e.*, by less than 1 per cent. on the whole body length.

The question as to the nature of these deleterious products of metabolism was attempted to be decided by allowing larvæ to develop in solutions containing varying proportions of uric acid and urea. The effects produced by these substances are shown in the table.

Number of experiment.	Substance present, and amount.	Effect produced.
		per cent.
79	Uric acid, 1 in 154,000	+ 5·3
80	„ 1 in 70,400	+12·2
122	„ 1 in 58,000	+ 5·8
121	„ 1 in 28,000	— 2·1
123	„ 1 in 76,400 (after first day)	+ 5·0
124	Urea 1 in 65,000	+ 2·3
159	„ 1 in 59,000	+ 3·7
81	„ 1 in 44,000	+ 2·2

Here we see that, so far from their exerting an injurious influence, urea, and especially uric acid, cause a considerable increase in the size of the larvæ. A solution containing 1 part of uric acid in 70,400 in water appears to be the most favourable, and it is only when the proportion is raised to 1 in 28,000, or when the water is more than half saturated with the uric acid, that an injurious effect is produced. It seems probable that the larvæ would develop without much diminution in size in an absolutely saturated solution (*i.e.*, one containing 1 in 15,000) of uric acid. In Experiment 123 the uric acid was added to the water at the end of the first twenty-four hours. The effect produced is considerably smaller than in Experiment 80, where a similar proportion of uric acid was present during the whole period of development. The beneficial effect of urea on development is not so marked, but it is nevertheless not to be denied. This remarkable influence of uric acid and urea on development at first sight seems contradictory to the conclusions arrived at experimentally for the higher animals. Thus, it is well known that the injection of a solution containing products of metabolism into a contracting muscle tends to decrease its contractility, and exhaust it, but, as far as I know, it has not been shown that the injection of small quantities of uric acid and urea have a harmful effect. No doubt the injection of considerable quantities would be injurious, just as when, in the above experiments, the proportion of uric acid present is increased to 1 in 28,000, a decrease in the size of the larvæ is brought about. In the light of these results on the development of larvæ, it seems possible that previous ideas upon the effects of moderate doses of products of metabolism such as uric acid and urea upon living tissues, may be erroneous. The growth of tissues following on their functional activity is considered to be partly the result of the increased blood supply, but this is generally admitted to be scarcely a sufficient cause. May not the presence of the products of metabolism in the tissues act as a stimulating influence, and by reaction bring about increased anabolic changes ?

In all the above experiments, impregnation was performed under normal conditions. A few observations were made in which it was effected in water in which larvæ had previously developed, and in water containing uric acid, but little or no measurable effect was produced. In Experiments 84 and 127 the ova were kept for an hour during impregnation in water in which larvæ had previously developed. The resulting larvæ varied by + 4·2 and + ·1 per cent., or, on an average, by + 2·1 per cent., from those grown under normal conditions. In Experiments 85, 128, 162 and 197 the ova were impregnated in water containing from 1 in 58,000 to 1 in 79,400 of uric acid. The larvæ varied respectively by + 7·6, — 5·3, + 2·3 and — 2·4 per cent., or on an average by + ·6 per cent. from those grown under normal conditions. As these mean variations produced in the size of larvæ impregnated in uric acid and previous fertilisation water are small enough to be within the limits of experimental error, it must be considered as probable that the protoplasm of the ovum and spermatozoon,

at the time of impregnation, is not specially sensitive to the presence of products of metabolism, as it is to the temperature and salinity of the water.

As calcium salts form such a considerable proportion of the body weight of the larvæ, it was thought possible that the injurious effects of water in which larvæ had previously developed, might be in some degree due to a small part of these salts having been absorbed by the larvæ, and so the supply rendered insufficient. In this case the presence of an additional amount of calcium salts might be expected to exert a beneficial effect on the development. Sea-water contains per litre about ·8 grm. of calcium salts, mostly as chloride. In Experiments 119, 136 and 137 were added to the water respectively ·0493, ·0804 and ·0512 grm. of calcium chloride per litre. The mean variations produced in the larvæ were − 3·1, + 1·2 and + ·8 per cent., or on an average −·4 per cent. As the amounts of calcium salts added are far greater than could be absorbed by ten or twenty thousand developing larvæ per litre, it follows that their absorption of calcium salts from the water has no appreciable influence on their growth.

The Effect of Dissolved Gases on Development.

The composition of the gases dissolved in sea-water is known to vary considerably, and as the developing larvæ respire through the medium of these gases, changes in their composition might be expected to produce some effect on the development. In Experiments 133, 141 and 179 the larvæ, after impregnation of the ova under normal conditions, were allowed to develop in sea-water which had previously been subjected to the action of an efficient water pump for about half-an-hour. The vessel containing the water was shaken up from time to time, to aid the liberation of the gases, so that a considerable proportion of them were withdrawn. Gases would be slowly absorbed again by the water from the air, but, as an analysis showed, the normal proportion was not wholly restored, even at the end of the eight days. The mean size of the larvæ varied by − 1·7, − 3·4 and + 1·1 per cent. respectively, or on an average − 1·3 per cent. This apparent decrease in size may be due only to experimental error, as it is so small, but it is more probably an actual effect of the diminished supply of gases.

In the observations on the effects of an increase in the amount of carbonic acid dissolved in the water, various volumes of sea-water, which had been saturated with carbonic acid gas, were mixed with normal sea-water. The carbonic acid, produced by the action of hydrochloric acid on marble, was purified by passing it through a wash-bottle of water, and was then allowed to bubble for an hour or more into the sea-water through an inverted funnel, whereby a larger surface of gas is exposed. In water containing 500 cub. centims., 350 cub. centims. and 200 cub. centims. of this carbonic acid water per litre, no larvæ would develop, but they grew successfully if

the proportion were reduced to 175 cub. centims. or less. The following were the variations produced in their size after eight days' development.

Number of experiment.	Volume of carbonic acid water per litre.	Effect produced.
	cub. centims.	per cent.
149	75	+1·5
166	75	+ ·2
142	90	+2·7
148	150	+1·5
165	175	−1·1

It will be seen that, so far from the larvæ being diminished in size, as would be naturally expected, there is a slight increase, if the proportion of carbonic acid water be kept down to 150 cub. centims. per litre. The mean increase in the first four observations is 1·5 per cent., and, in all five observations, 1·0 per cent. It seems very remarkable that, when the proportion of carbonic acid is kept only just low enough to allow the larvæ to develop at all, little or no diminution in their size is produced. This conclusion is of especial interest in connection with the results obtained for larvæ developed in water containing uric acid and urea, and would seem to confirm the view that certain products of metabolism tend to stimulate the tissues to increased, rather than to diminished activity.

In order to observe the effect on the development of the larvæ of an increased amount of oxygen, a current of oxygen, prepared by heating potassium chlorate and manganese dioxide, and purified by passing through dilute potash, was allowed to bubble through the water for an hour or more. In Experiments 180 and 191 the water was previously deaërated by the water pump, so that more oxygen might be absorbed. The variations produced in Experiments 126, 180, and 191 were respectively − 2·9, + 1·5, and − 2·3 per cent., or, on an average, − 1·2 per cent. It would seem, therefore, that an increased supply of oxygen has, at any rate, not a favourable effect upon the developing larvæ. This is not to be greatly wondered at, as the water in all cases contains a plentiful supply of oxygen, and, as the larvæ are so small, the tissues would always have quite sufficient for their wants.

The effects of changes in the proportions of the dissolved gases at the time of impregnation were scarcely studied at all. No larvæ developed from ova impregnated in water saturated with carbonic acid, whilst with water containing 350 cub. centims. of carbonic acid water per litre a few larvæ developed, but not sufficient for measurement. In Experiment 146 the ova were impregnated in water containing 1 in 4 of carbonic acid water. The larvæ are 2·1 per cent. smaller than the normal. In Experiment 129, where the impregnation was performed in oxygenated water, the larvæ were apparently 3·5 per cent. smaller.

As the water in which the larvæ are allowed to develop remained unchanged during the whole period of eight or more days, it was thought to be of interest to examine to what extent the composition of the dissolved gases varied during the time. Thus the developing larvæ are constantly absorbing oxygen and excreting carbonic acid, whilst fresh oxygen is constantly being absorbed by the water from the atmosphere. In order to determine the dissolved gases in the sea-water, a strong flask connected with an inverted condenser was rendered as completely free from air as possible by the water pump. 10 cub. centims. of water had been previously poured into the flask, as the water vapour evolved from this helps to sweep out the gases on evacuation. 350 cub. centims. or 400 cub. centims. of the sea-water of which the gases were to be determined were then allowed to enter the flask. This water was then boiled for twenty minutes to half-an-hour, under the greatly reduced pressure still maintained in the flask. Distilled water which has just ceased from boiling was now admitted into the flask, and the small volume of gases which had been boiled off from the sea-water was driven over into a PETTERSON'S gas analysis apparatus, where it was analysed exactly. In the table are shown the volumes of gases, at 0° C. and 760 millims. pressure, present in a litre of the various specimens of water examined. In several of these determinations dilute sulphuric acid was placed in the boiling-out flask. Thus DITTMAR, after a long series of test experiments,* has shown that, if no acid be added, only a variable and inconstant amount of the carbonic acid is driven off on boiling, though this is not the case with the oxygen and nitrogen. It is necessary to add acid to decompose the, for the most part unstable compounds the carbonic acid forms with the salts in solution.

Number of experiment.	Description of water.	Volume of gas per litre.	Volume of carbonic acid.	Volume of oxygen.	Volume of nitrogen.
		cub. centims.	cub. centims.	cub. centims.	cub. centims.
1	Water fresh from tank ($+H_2SO_4$) .	79·30	63·92	4·23	11·15
2	,, ,, ,, ($+H_2SO_4$) .	81·70	65·12	3·42	13·16
3	,, ,, ,,	21·77	6·44	2·88	12·44
4	Water from larvæ, 4th day	19·52	3·28	4·87	11·37
5	,, ,, 7th ,, ...	21·76	4·47	5·25	12·04
6	,, ,, ,, ,, ...	21·26	4·26	5·39	11·61
7	,, ,, 8th ,, ($+H_2SO_4$)	75·03	58·81	5·20	11·02
8	Oxygenated water, 4th ,, ...	18·47	2·84	5·04	10·59
9	Deaërated water, 8th day ($+H_2SO_4$)	70·68	54·44	4·55	11·69
10	Carbonic acid water (150 cub. centims. per litre) $+H_2SO_4$	88·88	74·44	4·19	10·25

In the first three experiments are shown the gases contained in specimens of water taken at different times from the large tank of sea-water. It will be seen that the

* *Ibid.*

amount of oxygen present varies considerably. In Experiment 4, water in which 4100 larvæ per litre had been developing for four days was analysed. The larvæ were got rid of by the addition of a small quantity of corrosive sublimate, which caused them to sink to the bottom of the jar. We see that the amount of oxygen present is considerably larger than in water fresh from the tank. In Experiments 5, 6, and 7, waters in which larvæ had developed 7, 7, and 8 days respectively, were examined. The amount of oxygen present is still greater than in the former case. Hence we see that the absorption of oxygen from the water by the developing larvæ is so slight, that it is much more than counterbalanced by the absorption of oxygen from the air. As the water in the jars has a much larger surface exposed to the air, in proportion to its bulk, than the water in a large tank, it rapidly becomes more oxygenated. These determinations also seem to show that the proportion of carbonic acid decreases in water containing the developing larvæ. This would be owing to its diffusion from the water into the air. In Experiment 8, some of the oxygenated water in which the larvæ of Experiment 180 had developed for four days, was examined. On comparing the amounts of gases present with those in the water of Experiment 4, which was also examined after the larvæ had developed four days in it, we see that the proportion of carbonic acid is smaller, and that of the oxygen greater, in spite of the four days during which diffusion to and from the air had been taking place. In Experiment 9, some of the deaërated water in which the larvæ of Experiment 133 developed was analysed. On comparing the figures with those of Experiment 7, we see that the actual volume of gas obtained from the water is 5·8 per cent. less, the diminution being chiefly due to loss of carbonic acid and oxygen. In Experiment 10, water to which 150 cub. centims. per litre of sea-water saturated with carbonic acid had been freshly added, was analysed. The amount of carbonic acid is 15·4 per cent. greater than that found in Experiments 1 and 2, with normal water. It is not remarkable, therefore, that the larvæ were killed when the proportion of carbonic acid water added was much greater than this.

In all the experiments thus far described, the water containing the developing larvæ was stirred up once a day. This was thought to be necessary, as the larvæ generally float together in masses near the surface of the water, so that the products of metabolism might collect and exert some influence on their development. In Experiments 83, 125, and 189, however, the water was left unstirred during the whole period of development. The larvæ vary by respectively $-3{\cdot}2$, $+3{\cdot}0$, and $+{\cdot}5$ per cent., or on an average by $+{\cdot}1$ per cent., from those grown under normal conditions. Under natural conditions, the larvæ would be subjected to constant motion in the sea, and hence the absence of this constant motion in the artificial developments might possibly exert some influence. In order to test this supposition, an arrangement was made, consisting of a suspended lever, to one end of which was attached a vessel containing a syphon tube, and to the other end a large inverted funnel, which was

placed in the jar containing the developing larvæ. The water from the tap which supplied the tank was allowed to run into the vessel, which, by means of the syphon tube, automatically emptied itself about once a minute. The inverted funnel was thus moved up and down, and the water in the jar kept in motion. The water was only supplied to the tank for every alternate period of two hours, and, of course, the larvæ would be only kept in motion whilst the water was running. In Experiments 82 and 190 the water was thus stirred during the whole period of development, variations of + ·4 and — 2·9 per cent. in the size of the larvæ being produced. In Experiment 109 the water was only stirred during the first 24 hours, which is perhaps the most important time, as the fertilised ova do not become free swimming larvæ until about 20 hours after fertilisation. The resulting larvæ are ·2 per cent. smaller than the normal. Hence it appears that the size of the larvæ is not affected either by leaving the water entirely unstirred, or by keeping it in frequent motion during the period of development.

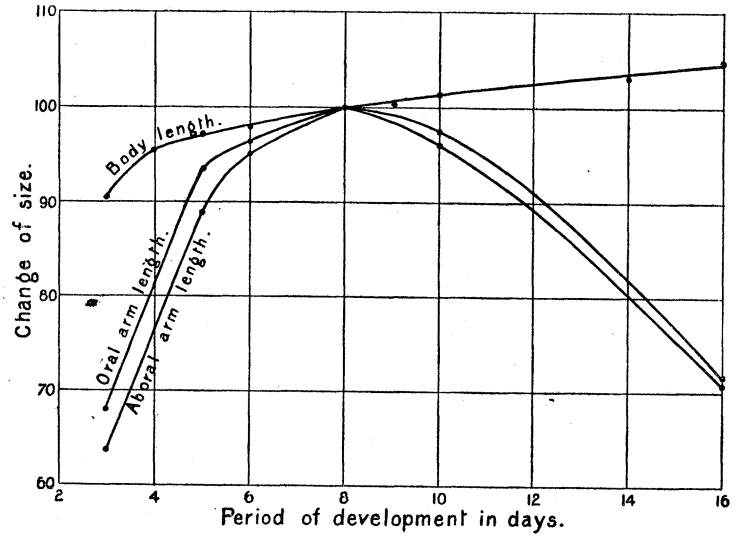
The Arm Lengths of the Larvæ.

We have thus far discussed the effects of environment only on the body length of the larvæ. As the lengths of the aboral and oral arms were also measured, it is possible to examine the effects of environment in their case also. The effects produced are not nearly so unequivocal as for the body length, but they are of interest for purposes of comparison. It will be seen that the same environmental condition need not by any means produce similar effects on both the body and arm lengths. It will first be necessary to examine how the lengths of the arms vary during the development of the larvæ. In the following table are shown the percentage variations in the body and arm lengths, at different times. The lengths after eight days' development are taken as 100. For the arm lengths, only values are given where the fertilisations contained less than 5500 larvæ per litre. In those experiments marked with an asterisk, the values are calculated, as corresponding observations were made only after six days' development, and not after eight.

Numbers of experiments.	Days of development.	Percentage variations in length of—			Mean length of—		
		Body.	Aboral arm.	Oral arm.	Body.	Aboral arm.	Oral arm.
94	3	−9·4	−36·2	−32·0	90·60	63·8	68·0
11*, 16*	4	−3·2, −5·1	..	..	95·85	..	..
13*, 17*, 95	5	−2·1, −4·9, −1·8	−11·3	−6·5	97·07	88·7	93·5
20, 23, 25, 28, 32, 38, 61, 68, 86, 96	6	−2·8, −·8, −·2, −5·6, −2·0, −4·2, −3·5, −3·7, +·2, +·2	−8·0, +3·7, −10·1, −·6, −11·2	−4·3, −2·4, −5·9, +1·2, −8·1	97·76	94·8	96·1
21, 24, 26, 29, 33, 39, 62, 69, 87, 97	8	..	..	..	100·00	100·0	100·0
22, 27, 30, 88, 98	10	+·1, +·3, −·6, +1·6, +1·1	+1·3, −10·1, −5·2, −2·6	−·7, −·5, −4·4, −4·9	101·10	95·9	97·4
31, 34	14	+2·8, +3·3	..	..	103·05	..	..
89, 99	16	+3·0, +6·5	−24·8, −33·3	−21·0, −36·2	104·75	71·2	71·4

In Fig. 6 these results are shown graphically. Here we see that the body length of the larvæ increases steadily, but in a diminishing degree, during the course of the

Fig. 6.



development, and exhibits no break at any point. The arm length, however, reaches a maximum after eight days' development, and from this point decreases again almost as rapidly as it had increased. This is due to the arms undergoing absorption previous to the larvæ passing into the stage where they are no longer free swimming. It is now evident why it was thought best in the great majority of instances to measure the larvæ after eight days' development.

As has already been shown, the number of larvæ developing in a given volume of water has, within wide limits, no practical influence upon their size. The effect

upon the arm length is, however, strikingly large. In the table, the means of the arm lengths in solutions containing different numbers of larvæ are given.

Number of different observations.	Number of larvæ per litre.	Mean length of aboral arm.	Mean length of oral arm.	Ratio of mean arm lengths.
19	Under 750	120·5	119·8	1·01
18	750 to 1,500	121·8	116·9	1·04
22	1,500 „ 2,500	111·6	110·0	1·01
10	2,500 „ 3,500	119·2	111·6	1·07
12	3,500 „ 4,500	104·4	100·8	1·04
9	4,500 „ 6,000	107·6	101·2	1·06
17	6,000 „ 8,000	106·4	101·7	1·05
17	8,000 „ 11,000	99·5	97·1	1·02
15	11,000 „ 15,000	100·8	96·0	1·05
12	15,000 „ 20,000	89·3	91·9	·97
4	20,000 „ 25,000	98·3	92·4	1·06
2	25,000 „ 30,000	60·0	74·1	·81
2	Over 30,000	56 6	68·5	·83

In the first column of the table are given the numbers of sets of measurements of which the values in the third and fourth columns are the means. Only measurements of larvæ which have developed for eight days are included. It will be seen that the length of the arms decreases in a more or less proportionate relation to the number of larvæ per litre. With 4000 larvæ per litre the arms are respectively 13·4 and 15·9 per cent. shorter than with only 500 per litre; with 17,500 larvæ, 25·9 and 23·3 per cent. shorter; whilst in the two observations made with over 30,000 they are 53·0 and 43·2 per cent. shorter. As the values in the last column of the table, which denotes the relations of the arm lengths, are within the limits of experimental error constant, it would seem that on an average the lengths of both the arms are affected by the number of larvæ per litre to an equal degree. Though the mean values of the arm lengths are more or less regular, the individual numbers show wide variations. In Figs. 7 and 8 are shown graphically the relations of the arm lengths of the larvæ, after eight days' development, to the numbers per litre. The crosses show the mean values which are given in the table, whilst the curved lines indicate the mean relation of arm length to number of larvæ per litre, as calculated by a formula which will be given shortly.

This marked influence on development of the number of organisms growing in a given volume of water has been noticed for other animals as well. KARL SEMPER* allowed *Lymnaeus stagnalis* to develop in different volumes of water, all the other conditions, as of nutrition and temperature, being the same. The shell of the mollusc which developed in 100 cub. centims. of water was 6 millims. in length; that

* "Die Natürlichen Existenzbedingungen der Thiere," 198.

Fig. 7.

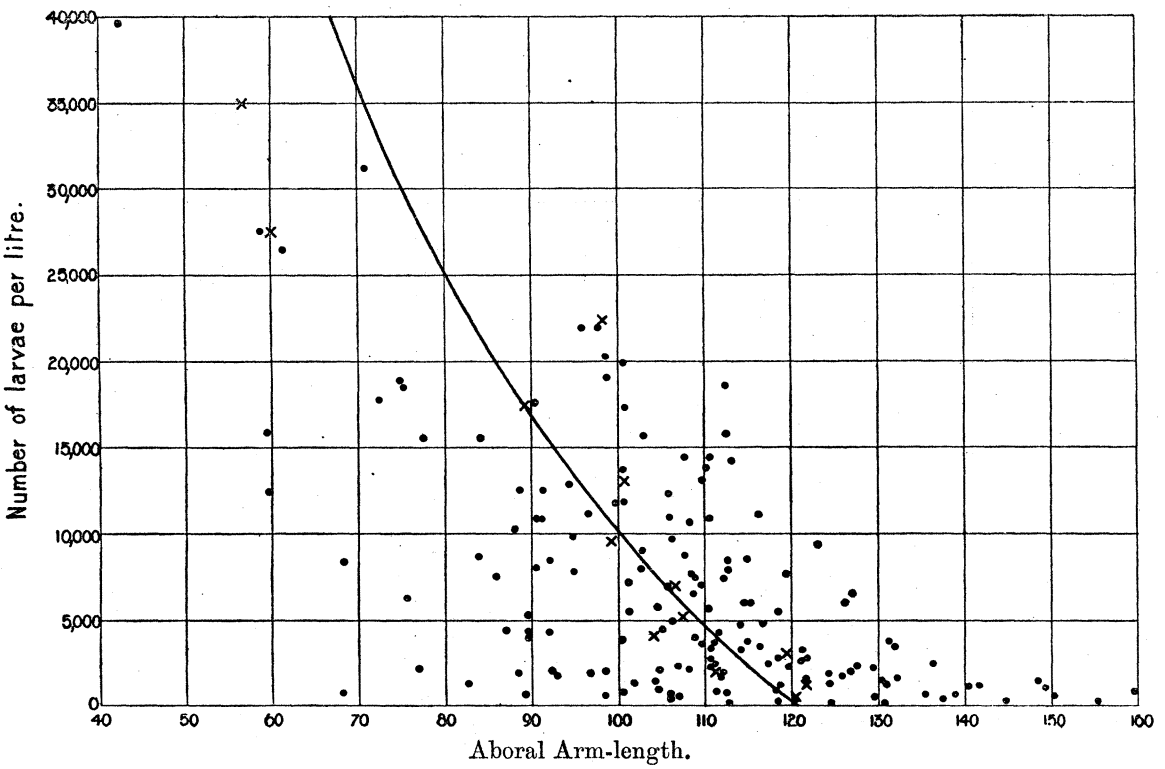
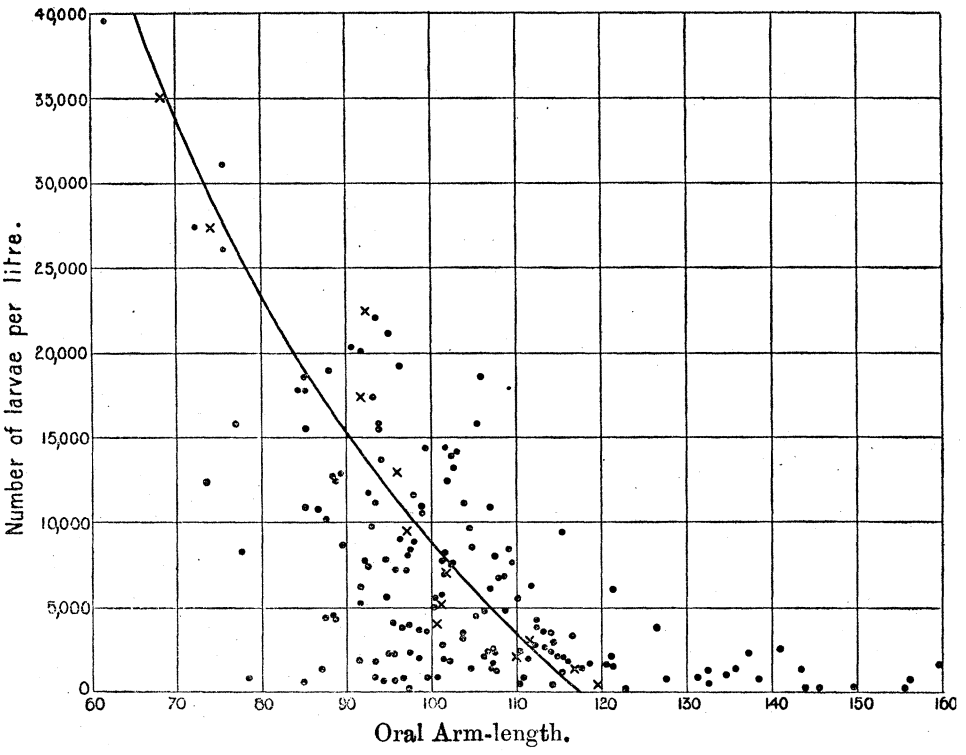

Number of larvae per litre.
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
40
50
60
70
80
90
100
110
120
130
140
150
160
Aboral Arm-length.

Fig. 8.


Number of larvae per litre.
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
60
70
80
90
100
110
120
130
140
150
160
Oral Arm-length.

in 600 cub. centims. of water, 12 millims; and that in 2000 cub. centims. of water, 18 millims. This decrease in size is in all probability due to the poisonous influences of certain of the products of metabolism. In the case of plutei, we have seen that it cannot be due to the presence of an excess of urea, uric acid, or carbonic acid, but there are other products, such as certain albumoses and other nitrogenous bodies, which, though present in even very minute quantities, might account for the effect noticed.

We have seen that in fertilisations containing less than 5500 larvæ per litre, the aboral and oral arm lengths reach a maximum at the eighth day. Their lengths on the sixth day are on an average respectively 94·8 and 96·1, the lengths on the eighth day being taken as 100. In fertilisations containing larger numbers of larvæ, the absorption of the arms seems to take place rather earlier. In Experiments 23, 28, 32, 61, and 68, where 7400 to 26,300 larvæ per litre were present, the lengths of the arms on the sixth day are on an average respectively 97·8 and 96·9. Again, the average lengths of the arms of larvæ in fertilisations containing under 5500 per litre, are after ten days' development respectively 95·9 and 97·4; but in Experiment 30, where there were 7400 larvæ per litre, their lengths are respectively 90·9 and 97·2. Hence it appears that in fertilisations containing the larger numbers of larvæ, the arm lengths increase less between the sixth and eighth days, and decrease more between the eighth and tenth days, than in those containing smaller numbers.

As it is desirable to study the effect of environment on the arm lengths of the larvæ, it is necessary to get rid as far as possible of this influence of number per volume of water. This has been done by calculating, by means of an empirical formula, the arm lengths which larvæ in a fertilisation containing x larvæ per litre, would have at infinite dilution. The formula found by trial to agree best with the various observations is—

$$\text{Arm length} \times \frac{10{,}000 + (\text{number of larvæ per litre} \times \cdot 2)}{10{,}000} = \text{const.} \begin{cases} = 120{\cdot}56 \text{ for aboral arm length} \\ = 117{\cdot}62 \text{ for oral arm length} \end{cases}.$$

In the table at the end of the paper are given the arm lengths of the larvæ reduced by means of this formula to their lengths at infinite dilution. Values have only been calculated for larvæ which had developed for eight days. The curved lines in Figs. 7 and 8 represent the mean relation of arm length to number of larvæ, as calculated from this formula. It will be seen that the crosses, which represent the mean arm lengths, are fairly well distributed about them.

If we glance at the corrected values for the arm lengths in the table at the end of the paper, it will be noticed that they frequently show great variations among each other, and that for no apparent cause. The arms seem to be exceedingly sensitive during development to slight changes of environment, such as have scarcely any influence on the growth of the body. In order to arrive at general conclusions as to the effects of the temperature of the water and the season, it is necessary to take

means of the arm lengths of the several sets of measurements made at each fertilisation. In the table these mean values are given. The mean body lengths, the mean temperatures during development, the mean numbers of larvæ per litre, and the mean "variabilities," which will be discussed later on, are also given. Only observations made after eight days' development are included. The mean aboral arm lengths vary between the extremes of 92·4 and 136·0; the oral between 93·0 and 147·5. As a rule, however, the oral arms vary in the different fertilisations less than the aboral, for the mean error in the one case is ± 8·0, and in the other ± 9·8.

Date.	Number of observations.	Mean						
		Aboral arm length.	Oral arm length.	Ratio of arm lengths.	Temperature of development.	Number of larvæ per litre.	Body length.	Variability.
					°			
April 24 . . .	2	121·6	118·7	1·02	16·0	5,950	34·42	16
„ 25 . . .	6	92·4	105·5	·88	16·0	16,075	32·91	23
May 9 . . .	5	127·9	120·6	1·06	17·2	1,650	34·93	26
„ 12 . . .	3	135·1	126·0	1·07	17·8	9,100	33·64	20
„ 17 . . .	4	107·0	111·2	·96	19·3	4,210	34·07	31
„ 21 . . .	6	120·2	116·6	1·03	19·1	2,130	32·85	23
„ 25 . . .	8	104·1	109·4	·95	18·2	6,400	32·61	28
„ 29 . . .	7	97·0	110·4	·88	18·7	16,400	32·59	25
June 4 . . .	11	125·7	119·5	1·05	19·5	9,950	30·44	30
„ 8 . . .	5	114·1	108·5	1·05	19·1	2,770	32·81	24
„ 13 . . .	11	119·5	109·5	1·09	18·7	3,580	30·41	23
„ 17 . . .	10	123·4	117·7	1·05	19·5	10,480	30·37	28
„ 22 . . .	10	115·6	111·9	1·03	20·8	5,850	33·15	23
„ 29 . . .	5	136·0	122·3	1·11	21·8	1,650	30·37	28
July 11 . . .	3	108·7	93·0	1·17	23·3	500	29·64	25
„ 25 . . .	9	113·4	105·6	1·07	23·7	1,070	27·33	27
August 7 . .	5	134·7	126·5	1·06	23·1	6,000	27·65	21
„ 7 . .	5	125·9	119·9	1·05	19·9	2,000	28·78	22
„ 10 . .	7	136·0	147·5	·92	22·5	700	27·54	25
„ 25 . .	6	131·4	136·5	·96	23·3	1,930	29·88	27
„ 25 . .	6	118·1	118·8	·99	20·7	2,000	31·43	25
September 14 .	12	131·0	119·5	1·09	22·2	13,400	34·52	24
„ 25 .	10	127·6	124·2	1·03	20·7	13,300	30·48	25
October 8 . .	3	127·3	125·7	1·01	17·8	17,400	33·95	20

The Effect of Temperature on the Arm Lengths.

It appears that, as a rule, the length of the arms increases with increase of the temperature of the water during development. Moreover, the aboral arm length increases more rapidly than the oral arm length, and hence the ratio between the two also increases. In the table we see that the temperature of the water increases more or less steadily, till it attains a maximum in the middle of July. It remains more or less constant at this point till the end of August. The arm lengths attain a maximum at about the middle of August, and the ratio between the arm lengths a maximum at

the middle of July. In order to render the relation between temperature of water and arm lengths more clear, the observations are still further reduced to mean values in the subjoined table :—

Number of observations.	Temperature of development.	Mean				
		Aboral arm length.	Oral arm length.	Ratio of arm lengths.	Number of larvæ per litre.	Body length.
21	15°·7 to 17°·9	116·1	116·4	0·998	9520	33·70
64	18·0 „ 19·9	115·6	113·5	1·018	8460	31·25
31	20·0 „ 21·9	123·2	118·5	1·032	6830	31·51
43	22·0 „ 23·7	128·1	123·1	1·041	5120	30·19

There are given the mean arm and body lengths, arm ratio, and number of larvæ, of all the observations in which the temperature of development lies below 18°, between 18° and 20°, 20° and 22°, and above 22°. It will be seen that the lengths of both the aboral and oral arms increase regularly with the temperature, from 18° to 20° upwards. In forty-three observations at temperatures above 22°, the aboral and oral arms are respectively 10·8 per cent. and 8·5 per cent. longer than those in the sixty-three observations made at 18° to 20°. At temperatures below 18°, the arm lengths seem to again increase. The ratio between the arm lengths increases steadily with the temperature, it being 4·3 per cent. higher at temperatures above 22° than at those below 18°.

Though a rise in the temperature of the water during development of the larvæ is accompanied by an increase in their arm lengths, yet it does not necessarily follow that the one is the cause of the other. There is, nevertheless, a great probability that such is the case in the present instance. Of the various possible errors which might lead to a wrong conclusion, one might arise through wrong corrections being applied in getting rid of the influence on the arm length of the number of developing larvæ. As the mean numbers only vary from 5120 to 9520 larvæ per litre, any error from this source would be too slight to produce the effect noticed. Again, as the arm lengths are all percentages on the body lengths, and as the body lengths tend to decrease with increase of temperature, it might be possible that the arm lengths, not being affected by the temperature to the same degree, should apparently increase in length on rise of temperature. The fact that the larvæ developed at 20° to 22°, which have considerably greater arm lengths than those developed at 18° to 20°, have also a slightly greater body length, proves that this is not the case. This same fact proves that the relative maturity of the ova and spermatozoa can account but in small part for this increased arm length at higher temperatures. Thus, the mean body length may be taken as an approximate index of the maturity, and, though

the period of least body length corresponds with that of the highest temperature during development, yet, as we have just noticed, the inverse relation between the two at other temperatures is not by any means exact.

A few experiments were made in which ova from the same sea-urchins were allowed to develop at different temperatures, and in which, therefore, none of the effects produced can be due to differences in maturity. These entirely confirm the conclusions drawn from the above table.

Number of experiments at		Number of observations at		Mean length of				Mean ratio of arm lengths at	
				Aboral arm at		Oral arm at			
20°.	23°.	20°.	23°.	20°.	23°.	20°.	23°.	20°.	23°.
52, 57	53, 54	2	2	110·7	142·0	113·7	127·7	·97	1·11
139	138, 140 to 146	1	8	107·7	114·1	107·5	105·2	1·00	1·08
152 to 156	147 to 151	5	5	125·9	134·7	119·9	126·5	1·05	1·06
170 to 175	164 to 169	6	6	118·1	131·4	118·8	136·5	·99	·96
Mean values				115·6	130·6	115·0	124·0	1·00	1·05

Thus it is seen that the arm lengths of the larvæ developed at 23° are respectively 31·0 per cent. and 7·8 per cent. longer than those developed at 20°, whilst the ratio is increased by 5 per cent. at 23°. These values are very similar to those given in the former table, where means of all the observations are taken.

When discussing the effect of temperature on the body length of the larvæ, it was noticed that an increase in the temperature during development from 20° to 23° caused about 2 per cent. *decrease* in size. Where the temperature fell below 18°, there was also a decrease in the body length. We arrive, therefore, at the interesting fact that *one and the same change in the environment produces a different effect in each of the parts of the larva subjected to measurement.* It causes a decrease in the body length, a considerable increase in the aboral arm length, and a slighter increase in the oral arm length. This is in some ways a remarkable conclusion, for it follows that all the tissues of the body of a comparatively simple organism like the pluteus are never at one and the same time adapted in the most favourable manner possible to their environmental conditions. If a condition is favourable to the growth of one portion of the tissues it is unfavourable to the growth of another portion.

It has been shown that the body length of the larvæ is reduced by some 4 or 5 per cent. by keeping the ova at about 8° or 25° during the time of impregnation. It appears that the arm lengths are affected to a larger extent even

than the body length. Thus as a mean of the fourteen experiments cited, in which the impregnation was performed at about 8°, the aboral and oral arms are on an average 8·5 per cent. and 8·0 per cent. shorter than those of the larvæ fertilised under normal conditions. As a mean of the nine experiments in which impregnation was performed at about 25°, the arms are respectively 2·9 per cent. and 2·0 per cent. shorter. As these arm lengths are percentages on the body lengths, which we know to be about 5 per cent. smaller than those of the normal larvæ, it follows that the arms are actually about 13 per cent. smaller when the impregnation is performed at 8°, and about 7 per cent. smaller when at 25°. As far as can be gathered from the figures, the ratio between the arm lengths is not altered when the impregnation is performed at 8° or 25°.

The Effects of Other Conditions on the Arm Lengths.

We have seen that the body length of larvæ allowed to develop in water diluted within certain limits is considerably increased, whilst in more concentrated water it is, if anything, slightly decreased. In the table are shown the percentage variations

Number of experiment.	Salinity of water during development.	Variation produced in		
		Body length.	Aboral arm length.	Oral arm length.
	cub. centims.	per cent.	per cent.	per cent.
183	987·5	+ 5·0	+ 1·7	− ·8
154	975	+ 9·7	− 2·0	− 2·0
131	950	+15·6	− 6·1	−10·4
172	925	+10·2	− 9·8	−12·6
153	875	+ 4·9	−22·4	−26·8
92	850	− 7·0	+14·0	+15·3
171	850	− 1·6	+17·9	+ 5·3
173	1075	− 1·3	+ 7·0	+ 2·3
93	1090	− 1·8	+ 2·3	− 4·9
132	1100	+ ·7	+21·9	+10·9
135	1150	+ ·1	− 5·5	−17·3

in the size of the arm and body lengths in the larvæ grown in the different solutions. The figures show clearly that in water diluted within such limits as to produce an increase in the body length, a *decrease* in the length of the aboral and oral arms is at the same time brought about. On the other hand, it appears that when the water is so much diluted as to produce a decrease in the body length, it is accompanied by an increase in the arm length. In concentrated solutions, where the body length is, if anything, slightly decreased, the arm length appears to be slightly increased, but the figures vary too much for any definite conclusion to be drawn. In the first five

experiments in the table the body length shows on an average an increase of 9·1 per cent., whilst the aboral and oral arm lengths show a decrease of 7·7 per cent. and 10·5 per cent. respectively. In the next six experiments the body length shows a decrease of 1·8 per cent., and the aboral and oral arm lengths an increase of 9·6 per cent. and 1·9 per cent. respectively. Though there is not much numerical similarity between the increase or decrease of body length, and the corresponding decrease or increase of arm length in individual experiments, yet it seems possible that this may be due only to experimental error, as the average values are much more consistent. If it be so, it would follow that the arm lengths of the larvæ are practically uninfluenced by the salinity of the water, although the body length is so largely affected: for it must be remembered that the arm lengths are calculated as percentages on the body lengths.

In the five observations made on the effect of impregnating the ova in diluted water, the corrected aboral and oral arm lengths are about 5 per cent. greater than the normal, but, as the numbers vary considerably in the different experiments, nothing definite can be concluded. The values for the arm lengths of ova impregnated in concentrated sea-water are a little more consistent. In the six observations made the aboral arm lengths are respectively + 13·4, + 6·9, − 4·2, + 28·0, − 12·8, and + 19·4, or on an average 10·1 per cent. greater than the normal, and the oral arm lengths + 16·1, + 11·6, − 4·9, + 26·9, − 4·2, and + 20·3, or on an average 11·0 per cent. greater than the normal. This increase is too large to be due to experimental error, so we must conclude that impregnation of the ova in concentrated water causes a slight increase in the body length of the larvæ and a considerable increase in their arm length. This is a somewhat curious result, as we have already seen that the subsequent development of the larvæ in concentrated water scarcely affects the arm and body lengths at all.

As comparatively few observations were made upon the effect of light on development, it will not be possible in most cases to speak with any degree of certainty as to the effects produced upon the arm lengths.

Numbers of experiments.	Colour.	Percentage of change produced in the size of		Mean change of length of		
		Aboral arm.	Oral arm.	Aboral arm.	Oral arm.	Body.
73, 140, 150	semi-darkness	−26·5, −30·4, +3·5	− 17·5, − 19·2, + 12·0	−17·8	− 8·2	+2·5
69, 91, 115, 161	absolute darkness	− 7·8, − 18·3, − 11·8, − 20·5	+3·9, −7·4, +2·1, − 7·6	−14·6	− 2·3	−1·3
72, 114	blue (copper sulphate)	+·5, −·1	+ 8·9, − 2·2	+ ·2	+ 3·3	−4·5
168, 181, 192, (116)	green	−5·0, −19·1, −21·9, (−7·2)	−16·4, −15·2, −·5, (− 3·7)	−15·3	−10·7	−4·8
160, 167	blue (Lyons blue)	− 29·0, − 25·7	− 18·4, − 18·8	−27·3	−18·6	−7·4
70, 112	red	− 3·3, + 8·2	+ 2·9, + 1·9	+ 2·4	+ 2·4	−6·9
71, 113	yellow	− 22·7, + 10·4	− 11·8, + 8·9	− 6·1	− 1·4	−8·9

In the table the percentage variations in the arm lengths are given, the mean effects produced on the body lengths being also added for purposes of comparison. It seems that the arm lengths are practically not affected by either the blue light of copper sulphate solution, or by red and yellow light, more than the body length is affected. Green light and the violet blue light of Lyons blue solution seem on the other hand to exert a markedly unfavourable influence, the decrease being too great to be accountable to experimental error. Though, as we have already seen, little or no deleterious effect is produced on the body length by growing the larvæ in absolute or semi-darkness, yet the arm lengths are considerably decreased. It would appear from the figures in the table that semi-darkness exerts a more adverse influence than absolute darkness, but this result may be due to incidental error. In the case of light, therefore, as in the cases of temperature and change of salinity of the water, a different effect is produced on the arm and body lengths by the same changed environmental condition.

As the arm lengths of the larvæ are so largely affected by the number growing in a given volume of water, it would be expected that when placed during the whole period of their development in water in which larvæ had already been allowed to develop, a very marked effect would be produced. Such is actually the case. In Experiments 57 and 76 the aboral arm length is diminished 22·2 and 41·6, or on an average 31·9 per cent., and the oral arm length 13·4 and 22·0, or on an average 17·7 per cent. In Experiments 77 and 78, where the larvæ were only placed in previous fertilisation water for a portion of the time of development, the aboral arm length is diminished by 24·0 and 32·7, or on an average 28·4 per cent., and the oral by 19·8 and 27·9, or on an average 23·9 per cent.

In the observations made on the development of larvæ in water containing uric acid

and urea, it appears that but little effect on the arm lengths is produced. If anything, they are slightly increased in length, but the values are not consistent enough to render their discussion of interest. Apparently, also, little or no effect upon the arm length is produced by impregnating the ova in solutions of uric acid or previous fertilisation water, and no effect seems to be produced by changes in the composition of the gases dissolved in the water. In those cases where such an additional amount of sperma was added as to render the water putrid, the arm lengths are considerably decreased, but otherwise they are probably not influenced either by a decrease or an increase in the amount present.

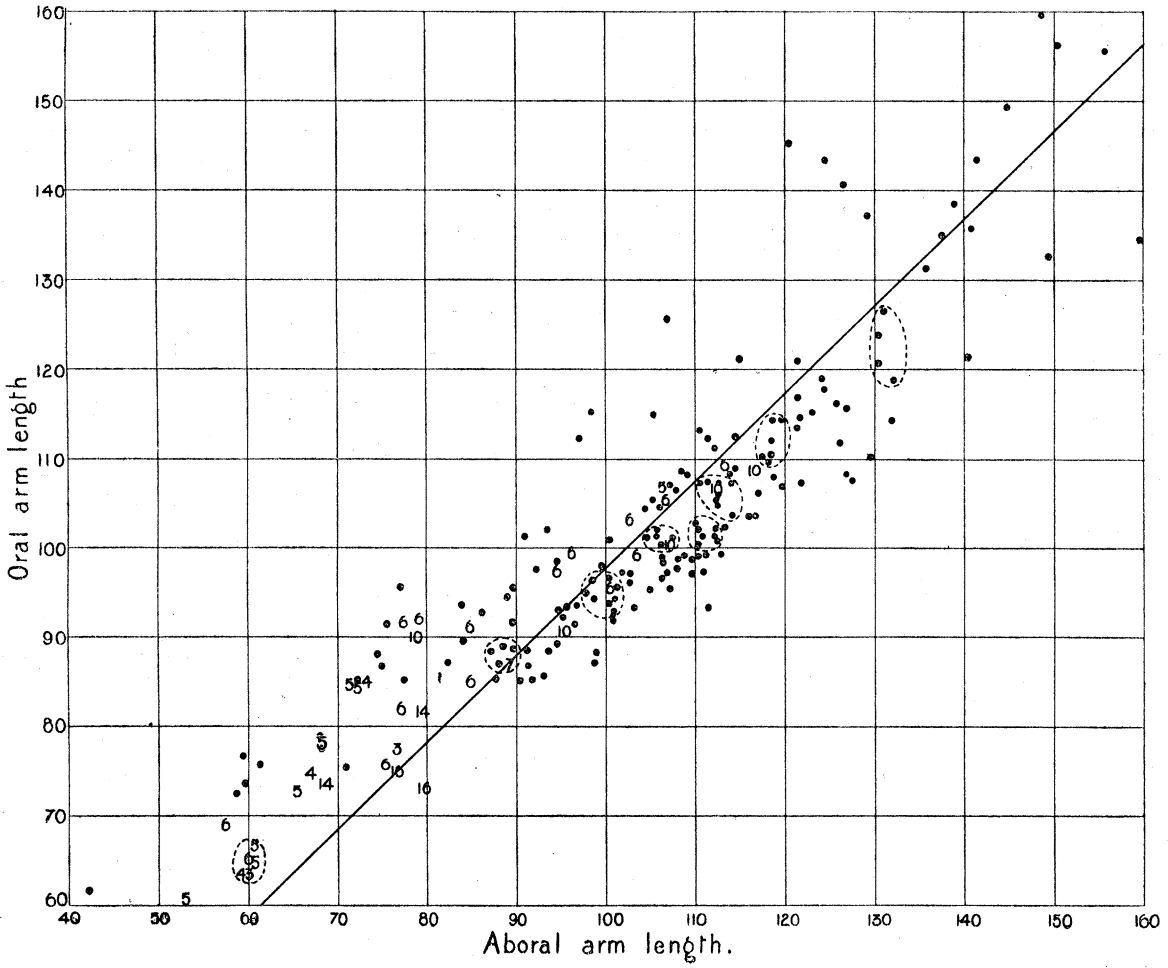
Though the evidence drawn from the variations produced in the arm lengths of the larvæ is not nearly so trustworthy as that for the body length, probably because of the greater sensitiveness of the tissues of the arms to slight changes in the environment, yet the number of different observations made in several cases affords a considerable degree of certainty to some of the conclusions arrived at. Probably the most striking point of these conclusions lies in the fact that opposite effects on the arm and body lengths are frequently produced by the same change of environmental condition. The effects brought about by rise of temperature and dilution of the water during development are especially noticeable.

Both the aboral and oral arm lengths of the larvæ were determined, in order that the effect of environment on the relation between the two might be examined. We have seen that in the case of an increased temperature during development, the oral arm length is not so much affected as the aboral, and hence, the ratio between the two is increased. In the other observations it may also be noticed that as a rule the oral arm length is not affected to so great a degree as the aboral. The reason of this is obvious. The measurement taken as to the oral arm length is made up in part of the true oral arm length, and in part of the body tissues, whereas the aboral arm length measurement is made up only in a very slight degree of the body tissues. As in most cases the body tissues are modified by changes of environment to a much smaller degree than the arm tissues, the oral arm length is the less affected.

The mean of all the numbers expressing the ratio between the arm lengths is 1·015. Of the 200 observations, 138, or 69 per cent., vary between the limits of ·92 and 1·10. The mean ratio of the 41 observations on larvæ allowed to develop less or more than eight days is ·951 ; the mean ratio of the 159 observations on larvæ developed in eight days, 1·025. In Fig. 9 the ratios of the arm lengths are shown graphically. The ratio is indicated by a dot where the period of development was eight days, and by a number—the number of days of development—where it was more or less than eight days. The straight line across the figure indicates the mean ratio of the arm lengths after eight days' development. The meaning of the small circles enclosing certain of the values will be rendered evident later on. The ratios are not distributed about the straight line as evenly as might be expected. This is accounted for partly by the changes in the environment to which the developing larvæ were subjected, and

partly by the variations in the ratio of the arm lengths in particular fertilisations. These latter are frequently very large. In the larvæ of the fertilisation on July 11, the mean ratio is 1·17. From this time it falls steadily, till by August 16 it is only

Fig. 9.



·92. The temperature of the water during development, and the body length of the larvæ, are practically the same in both instances, and, hence, this decrease of 21 per cent. seems to be entirely due to the individual peculiarities of the larvæ.

Statistical Examination of the Body-length Measurements.

As the number of measurements made was so considerable, it was thought to be of interest to subject them to statistical examination, though from their nature they are not very well suited to this purpose. The various sets of observations were arranged in a series, according to the magnitude of the body length, and then separated into groups, each comprising about 10 sets, or 500 larvæ. The extreme mean body lengths of these groups generally only differ by about ·5. The frequency of occurrence of deviations from the average in the body lengths of the larvæ in these

groups was then determined, according to the statistical method employed by GALTON in his work on 'Natural Inheritance,' and elsewhere. The values are sorted in order of magnitude, and the number of larvæ with, for instance, when the mean body length is about 30·0, a body length of 23, 24, &c., up to 37, determined. It is then possible to calculate what, according to GALTON's notation, is spoken of as the *median*, or M. This is the middle value of all the measurements, or the particular body length which has 50 per cent., or half the other values lower than itself, and half of them higher than itself. This value is spoken of as the body length at grade 50°. In the same way the body lengths at grades 25° and 75°, or body lengths such that 25 per cent. and 75 per cent. of the larvæ respectively are smaller than themselves, can be calculated. If the frequency with which the observed deviations from the average occur obey the law of error, then the "probable error" of the body length is represented by the difference of the body length at grade 50°, from that at grade 25°, and that at grade 75°. These values are denoted in GALTON'S notation by the symbols Q_1 and Q_3, and in normal cases they should be equal in magnitude and opposite in sign. In the observed cases there is usually a slight difference between the two, and, therefore, their mean value is taken as the probable error, and is denoted by the symbol Q.

In order to determine the correspondence between the frequency of the observed values, and that indicated by the probability integral or theoretically calculated curve of error, the body lengths at grades 5°, 10°, 20°, 30°, 40°, 60°, 70°, 80°, 90°, and 95°, were determined, in addition to those at grades 25°, 50°, and 75°. From these values the body length at grade 50° is subtracted, and the remainder divided by the probable error, or $Q_1 - Q_3/2$. Values are thus obtained at the various grades, which are multiples of the probable error. These values, in all cases where the deviations from the average obey the law of error, should be practically identical with the theoretically calculated values, which are given in the bottom line of the subjoined table. In this table are shown the results of the statistical examination of the body lengths of 9850 larvæ, or all the larvæ measured with the exception of three sets of observations with extreme values. These larvæ have been separated into eighteen groups, of about 500 to 600 each. In the third column are given the arithmetical means of the body lengths of the various groups of larvæ, and in the fourth column the median values *plus* ·5. In order to compare the median with the arithmetic mean, it is necessary to add ·5, for when the larvæ were sorted into groups with body lengths of 23, 24, &c., no notice was taken of the decimal figure, and hence, a body length of 23 is in reality one of 23·5, and one of 30, 30·5. In the fifth column it will be seen that M + ·5 is invariably lower than the arithmetic mean, it being on an average ·41 smaller. This is due to an error incidental to the method of taking arithmetical means. Thus the body lengths are sorted according to unit values, but in, for example, the above quoted instance, the unit difference between 23 and 24 is $\frac{1}{23}$ of the body length, or 56·5 per cent. greater in proportion to the body length than unit difference between 36

Length of body.	Number of larvæ.	Arithmetical mean length.	M + ·5.	Difference.	Q found.	Correction.	Corrected Q.	Corr. Q. / Arith. mean.	5°.	10°.	20°.	25°.	30°.	40°.	50°.	60°.	70°.	75°.	80°.	90°.	95°.
26·67 to 27·61	400	27·18	26·75	·43	1·80	−·23 + ·03	1·60	·059	2·68	2·08	1·28	1·01	·75	·36	·00	·35	·76	·98	1·22	1·90	2·59
27·68 „ 28·13	650	27·83	27·36	·47	1·80	−·11 + ·03	1·72	·062	2·54	1·90	1·27	1·02	·82	·41	·00	·38	·77	·98	1·19	1·99	2·66
28·21 „ 28·62	350	28·42	27·94	·48	1·80	−·10 + ·03	1·73	·061	2·55	1·75	1·10	·96	·74	·37	·00	·36	·81	1·04	1·29	1·98	2·56
28·81 „ 29·21	400	28·99	28·62	·37	2·17	−·08 + ·04	2·13	·074	2·46	1·78	1·28	1·03	·81	·37	·00	·32	·76	·98	1·29	1·77	2·36
29·39 „ 29·91	700	29·66	29·35	·31	2·00	−·14 + ·04	1·90	·065	2·43	1·88	1·30	1·08	·84	·37	·00	·36	·73	·93	1·15	1·87	2·41
30·01 „ 30·41	950	30·21	29·72	·49	1·97	−·10 + ·04	1·91	·063	2·43	1·88	1·20	·97	·76	·38	·00	·41	·81	1·03	1·26	1·87	2·52
30·47 „ 30·63	600	30·56	30·19	·37	1·87	−·03 + ·03	1·87	·061	2·69	2·02	1·32	1·01	·78	·41	·00	·37	·73	·99	1·26	1·92	2·40
30·77 „ 31·20	550	31·07	30·62	·45	2·00	−·10 + ·04	1·94	·062	2·42	1·83	1·20	·96	·77	·37	·00	·41	·82	1·04	1·29	1·94	2·57
31·24 „ 31·64	700	31·46	31·06	·40	1·95	−·09 + ·04	1·90	·060	2·52	1·91	1·22	·99	·77	·33	·00	·35	·73	1·02	1·25	1·79	2·28
31·74 „ 32·11	500	31·96	31·67	·29	2·18	−·09 + ·04	2·13	·067	2·32	1·91	1·33	1·09	·83	·36	·00	·30	·69	·91	1·17	1·82	2·20
32·17 „ 32·66	500	32·43	32·03	·40	2·20	−·08 + ·04	2·16	·067	2·49	1·91	1·25	·99	·76	·36	·00	·37	·79	1·01	1·25	1·78	2·32
32·73 „ 33·11	500	32·97	32·62	·35	1·95	−·07 + ·04	1·92	·058	2·51	1·98	1·31	1·06	·86	·44	·00	·39	·74	·93	1·21	1·91	2·55
33·17 „ 33·45	600	33·29	32·91	·38	1·84	−·07 + ·04	1·81	·054	2·64	2·08	1·25	·98	·73	·34	·00	·33	·77	1·02	1·27	1·98	2·40
33·53 „ 33·79	600	33·67	33·27	·40	1·97	−·08 + ·04	1·93	·057	2·63	2·07	1·34	1·03	·77	·36	·00	·38	·77	·97	1·19	2·01	2·76
33·85 „ 34·17	550	34·02	33·61	·41	1·92	−·08 + ·04	1·88	·056	2·57	1·90	1·21	·98	·81	·43	·00	·44	·82	1·02	1·28	1·91	2·46
34·29 „ 34·79	600	34·52	34·01	·51	1·94	−·13 + ·04	1·85	·054	2·32	1·83	1·26	1·00	·75	·38	·00	·38	·75	1·00	1·28	2·09	2·68
34·91 „ 35·35	400	35·07	34·77	·30	2·06	−·11 + ·04	1·99	·057	2·74	1·97	1·29	1·06	·86	·41	·00	·38	·73	·95	1·21	1·80	2·23
35·39 „ 36·08	300	35·66	35·16	·50	2·39	−·15 + ·05	2·29	·064	2·31	1·86	1·16	·94	·74	·30	·00	·36	·82	1·06	1·31	1·95	2·35
Mean values	..	..	..	·41	1·99	..	1·93	·061	2·51	1·92	1·25	1·01	·79	·38	·00	·37	·77	·99	1·24	1·90	2·46
Theoretical values .	..	..	..	..	..	..	..	..	2·44	1·90	1·25	1·00	·78	·38	·00	·38	·78	1·00	1·25	1·90	2·44

and 37. The other unit differences vary from each other in a similar, though smaller, degree. In determining the arithmetic mean however, unit difference between 36 and 37 counts as much as unit difference between 23 and 24, and hence, too high a result is obtained. This source of error, on the other hand, has no influence on the medians.

As the mean body lengths in the several sets of observations in the different groups vary between different limits, a correction has to be applied to the probable error. This is done by subtracting from it the probable error of the several mean body lengths in each group, or what is practically the same thing, the mean error divided by 1·183. The numbers thus calculated and subtracted are given in the seventh column of the table. As, however, on an average any single observation of the body length differs from the median by the probable error, a series of fifty observations would differ by Q/50, hence this amount must be subtracted from the above correction. The numbers thus subtracted, or what comes to the same thing, added to the value of Q found, are also given in the seventh column. In the next column of the table the corrected values of Q are given, and in the next the relation of these values to the arithmetical mean body length. The rest of the table gives the observed coefficients at the different grades. These coefficients in all cases agree fairly well with the theoretical, and their mean values approximate very closely to them. They are, in fact, in several cases identical, whilst with the exception of the value at grade 5°, they none of them differ by more than ·02. When we remember the variable conditions under which the larvæ measured in these groups were allowed to develop, it is rather to be wondered that the variations in the coefficients are so small. There is thus no reason to doubt that whatever variations are produced in the average size of the larvæ by changes of environmental conditions, the frequency of the deviations in the size from the average always corresponds with that indicated by the theoretical law of error.

As the mean size of the larvæ in the various groups varies from 27·18 to 35·66, or by 31 per cent., the probable error would also be expected to show a certain amount of change. The mean corrected probable error of the first six groups of measurements in the table is 1·83, of the next six 1·99, and of the next 1·96. The mean relation of probable error to body length in the three groups of six determinations each is ·0640, ·0625, and ·0570 respectively. If the individual relative values in the table be considered, it will be seen that with one exception they remain at ·060 and upwards when the body length varies between 26·67 and 32·66, but that when the body length rises above these limits, the relative probable error undergoes a sudden drop, and with a single exception does not rise above ·058. Now larvæ developed under natural conditions probably rarely, if ever, would have a mean body length less than 32·73, for in these artificially developed larvæ, with one or two exceptions, the body length only fell beneath this limit when fertilisations were performed in the summer months, with not thoroughly mature ova. There seems, therefore, to be a considerable likelihood that the probable error is increased when larvæ are artificially developed under conditions which do not obtain, except perhaps in rare cases, in a state of nature.

That this supposition is correct is borne out by the fact that the relative probable error of the smallest larvæ, which developed under the most unnatural conditions, is greater than that for the medium sized larvæ.

As the larvæ were only measured in sets of fifties, it is not possible to determine the probable error in each set; but in order to get some idea as to the variability under different conditions of environment, the following method was adopted. The average probable error in each set of measurements was determined by multiplying the mean body length by ·061, *i.e.*, the mean relative probable error of all the groups of larvæ. The number of larvæ in each set of fifty was then counted, of which the body length was outside the limits of mean body length ± probable error. If the variability is exactly average, this number would be 25; if it is greater than the average, the number would be larger, and if smaller, smaller. In the last column of the table at the end of the paper, are given the numbers indicating the "variability" in the size of the larvæ in each set of measurements, as determined by this method. In the last column of the table on p. 606, are given the means of all the values for each fertilisation. Out of the twenty-four values, only six fall outside the limits of 22 to 28. Even this degree of variation is due partly to artificial causes. Thus it will be remembered that, in performing fertilisations, the ova of three different sea-urchins were, as a rule, impregnated with the sperma of three others. It might therefore happen that ova giving rise to larvæ of nearly similar or of very different size, were used in a fertilisation, and hence a small or a large probable error in the size of the mixed larvæ be brought about.

The variability seems to be affected considerably by the period of development of the larvæ. In the table are shown the mean "variability" values of all the observations made when the period of development was less or more than eight days. Of the observations made after eight days' development, only those are included where corresponding measurements were also made after six days' development.

Days of development	3	4	5	6	8	10	14	16
Number of sets of observations .	2	6	9	13	11	6	2	2
"Variability"	23·0	24·2	25·4	23·2	22·7	21·8	16·0	20·0

It appears that the variability increases regularly up to the fifth day, and then decreases regularly again, the values being very consistent, with the exception of that at 14 days. As most of the observations at 4 and 5 days were made on larvæ which were not also measured after eight days' development, they cannot be much trusted, but all the larvæ measured after ten days' development, were also measured after six and eight days, and hence it can scarcely be doubted that the variability does diminish

considerably as the period of development increases from six days. All the observations embodied in this table were made under normal, and as nearly as possible identical, conditions of environment; and hence, as we should be inclined to expect, the variability in the size of the larvæ appears to be within somewhat smaller limits than in the case of the observations made under changed conditions of environment. Thus we know that the average variability of all the observations is 25·0. The average variability of the observations in this table is only 23·0, whilst the average variability of all the observations made, with the exception of those included in this table, is 25·7. It may thus be looked upon as probable that change in the conditions of environment from the normal tends to increase somewhat the limits of variation in the size of the larvæ.

The temperature during development seems to act in the same way on the variability of the body length as it does on the actual body length. Thus, it will be remembered that a temperature of 18° to 20° is most favourable for the development of the larvæ; those above 22° or below 18° being least favourable. In 21 observations after 8 days' development at 16° to 18°, the mean variability is 22·2; in 64 observations at 18° to 20°, 26·3; in 31 observations at 20° to 22°, 24·8; and in 43 observations at 22° to 24°, 24·0. Thus the variability is greatest at the temperature most favourable for development, and smallest at those most unfavourable. The temperature has probably rather a greater effect on the variability than is denoted by these figures, for we have seen that with larvæ of small body length the probable error is proportionately greater than with the full-sized larvæ. Now most of the observations at 18° to 20° were made on full-sized larvæ, and most of those at 22° to 24° on small-sized larvæ, and hence a contrary influence would be acting on the variability. As far as can be judged, the variability is not affected by impregnation at low or high temperatures, but the values obtained are not very consistent, so no certain conclusions can be drawn. Also the variability does not appear to be affected at all by the number of larvæ developing in a given volume of water. The data are insufficient for determining the effects of light, salinity, and addition of urea and uric acid on the variability, but as far as can be judged from the few at hand, no effect is produced.

It is a question as to whether the variability in the body length measurements is affected by changes in the environmental conditions as readily as the actual body length. So far as can be judged from the few data available, it is more stable, but we have seen that it is at any rate affected by the season in which the fertilisations are performed, or by the maturity of the ova and sperma, by the period of development of the larvæ, and by the temperature of development. WELDON* found the probable errors of the four organs measured in the local race of shrimps obtained from Plymouth, to be some 25 per cent. greater than those of the same organs measured in shrimps obtained from Southport. This difference of variability is probably due to the differences in the environmental conditions of the two races. We can, therefore, conclude with certainty that the variability in the size of an organism is affected by changes of environment, as well as the actual size.

* 'Roy. Soc. Proc.,' vol. 47, p. 445.

The Arm Length Measurements.

Some of the measurements of the arm lengths of the larvæ were subjected to statistical examination, but they are not as well suited to this purpose as the body length measurements. Those sets of measurements were chosen, which had very nearly the same aboral arm lengths, and also nearly the same oral arm lengths. The body lengths generally varied considerably, as it was impossible to get sufficient sets of observations for measurement, of which the larvæ had nearly identical body, aboral arm, and oral arm lengths. The groups of measurements chosen are shown in Fig. 10 (p. 622), where they are enclosed in small circles. In all, eight different groups, comprising 2600 larvæ, were statistically examined. The larvæ were sorted into groups in which the arm lengths increased by five units at a time, *e.g.*, 100 to 105, 105 to 110, and so on. The results obtained are shown in the table. The first part gives the probable errors and coefficients of the aboral arm length measurements, and the second part those of the oral, similar "numbers of experiments" being for the aboral and oral arm lengths of the same larvæ. The mean relation of the corrected probable error to the mean aboral arm length is ·114, but the values vary very considerably in the different groups of measurements. They seem to reach a minimum with an arm length of about 100, and to increase as the arm length diminishes or increases, but this apparent variation may be due only to incidental error.

The relative probable errors of the oral arm lengths vary in an analogous manner, they also reaching a minimum with arm lengths of about 100. The medians differ from the arithmetical means by on an average 2·94 for the aboral arm lengths, and 2·70 for the oral arm lengths. These discrepancies are larger than in the case of the body length measurements, because the probable errors are greater. Thus the relation of mean difference between median and arithmetical mean to uncorrected probable error is for the body length ·20, for the aboral arm length ·24, and for the oral arm length ·26. The coefficients of the arm length measurements vary much more than did those of the body length, but this is no doubt in part due to the smaller numbers of larvæ subjected to measurement. Even the means of the coefficients of the aboral arm lengths show considerable discrepancies from the theoretical values. All the coefficients but one, for grades below 50°, are smaller than the theoretical, and all those above 50° are larger. Should this be taken to show that the larvæ are slightly dimorphic, with respect to their aboral arm lengths, in the same way as female specimens of *Carcinus mænas*, obtained from Naples, were found by WELDON* to be dimorphic with respect to their frontal breadth of carapace? The means of the coefficients of the oral arm lengths agree for the most part very well with the theoretical values, and hence, if there is any dimorphism in respect of the aboral arm lengths of the larvæ, there is no correlated dimorphism of the oral arm lengths.

* 'Roy. Soc. Proc.,' vol. 54, p. 318.

Number of experiments.	Arm length.	Number of larvæ examined.	Arithmetical mean.	M.	Difference between Arith. mean and M.	Q found.	Correction.	Corrected Q.	Corrected Q. / Arith. mean.	5°.	10°.	20°.	25°.	30°.	40°.	50°.	60°.	70°.	75°.	80°.	90°.	95°.
1	59·62 to 60·63	250	60·25	57·50	2·75	8·25	— ·30 + ·16	8·11	·135	2·25	1·88	1·26	1·00	·75	·35	·00	·39	·79	1·00	1·21	1·75	2·26
2	87·04 ,, 89·91	250	88·61	85·26	3·35	10·87	— ·72 + ·21	10·36	·117	2·39	1·90	1·12	·99	·71	·32	·00	·35	·80	1·02	1·28	2·13	2·91
3	98·03 ,, 101·13	500	100·06	96·38	3·68	9·80	— ·79 + ·18	9·19	·092	2·33	1·70	1·04	·82	·66	·34	·00	·39	·89	1·18	1·53	2·16	2·80
4	104·68 ,, 107·89	300	106·30	102·31	3·99	9·13	— ·67 + ·17	8·63	·081	2·72	1·73	1·03	·83	·64	·31	·00	·49	·93	1·15	1·45	2·16	3·30
5	110·03 ,, 112·61	350	111·25	107·86	3·39	12·95	— ·86 + ·25	12·34	·111	2·80	1·91	1·22	·96	·72	·33	·00	·45	·81	1·04	1·43	2·19	2·84
6	110·58 ,, 114·54	450	112·86	111·04	1·82	12·90	— ·84 + ·25	12·31	·109	2·70	2·01	1·24	·99	·80	·42	·00	·39	·89	1·01	1·21	1·85	2·29
7	117·76 ,, 119·85	300	118·62	115·83	2·79	18·16	— ·25 + ·36	18·16	·153	2·66	2·00	1·30	1·01	·60	·26	·00	·33	·76	·99	1·23	1·93	2·50
8	130·37 ,, 132·49	200	131·11	129·34	1·77	15·04	— ·58 + ·30	14·76	·113	2·28	1·88	1·41	1·07	·85	·43	·00	·33	·70	·93	1·09	1·87	2·42
	Mean values ..	..	..	..	2·94	12·14	.. ..	11·73	·114	2·52	1·88	1·20	·96	·72	·35	·00	·39	·82	1·04	1·30	2·00	2·66
1	63·33 to 66·86	250	64·36	61·67	2·69	7·40	— ·74 + ·14	6·80	·104	2·04	1·69	1·19	·96	·81	·35	·00	·38	·77	1·04	1·31	1·96	2·43
2	87·04 ,, 88·92	250	88·37	84·85	3·52	8·82	— ·56 + ·17	8·43	·095	2·58	2·08	1·29	1·03	·80	·30	·00	·30	·70	·97	1·47	2·43	3·13
3	92·76 ,, 96·83	500	94·94	91·60	3·34	8·88	— ·91 + ·16	8·13	·086	2·42	1·85	1·18	·92	·70	·32	·00	·42	·86	1·08	1·33	2·11	2·82
4	100·19 ,, 102·08	300	101·15	98·77	2·38	8·11	— ·54 + ·15	7·72	·076	3·01	2·31	1·33	1·06	·83	·38	·00	·34	·73	·94	1·21	2·15	2·87
5	100·61 ,, 102·85	350	101·88	98·82	3·06	10·02	— ·36 + ·20	9·86	·097	2·73	1·70	1·11	·94	·71	·36	·00	·44	·81	1·06	1·33	2·18	2·91
6	103·99 ,, 107·72	450	106·51	103·83	2·68	10·99	—1·01 + ·20	10·18	·096	2·81	1·94	1·22	·96	·75	·38	·00	·41	·79	1·04	1·32	2·02	2·63
7	110·11 ,, 114·28	300	111·99	109·90	2·09	15·28	—1·36 + ·28	14·20	·127	2·41	1·94	1·34	1·09	·80	·40	·00	·33	·67	·90	1·18	1·75	2·56
8	118·82 ,, 126·50	200	122·24	120·39	1·85	12·00	—2·02 + ·20	10·18	·083	2·67	1·98	1·33	1·07	·87	·49	·00	·45	·72	·93	1·15	1·80	2·36
	Mean values ..	..	..	..	2·70	10·19	.. ..	9·44	·095	2·58	1·94	1·25	1·00	·78	·37	·00	·38	·76	1·00	1·29	2·05	2·71
	Theoretical values..	..	..	..	..	..	.. ..	..	..	2·44	1·90	1·25	1·00	·78	·38	·00	·38	·78	1·00	1·25	1·90	2·44

In the case of one group of larvæ, the method introduced by GALTON,* and subsequently adopted by WELDON,† was applied in order to determine the correlation between the aboral and oral arm lengths. According to this method, the individual larvæ are sorted into groups, such that the aboral arm lengths, AC, are the same in each group. The mean length of the oral arm length, AD, in each group, is then determined. From each value obtained in this way is subtracted the respective median, and the difference is divided by the probable error of the aboral or oral arm lengths as the case may be. A series of pairs of values is thus obtained, which, according to the law of correlation, should have a constant relation, denoted by the symbol r, to each other. A group of 450 larvæ was subjected to this examination. Of their aboral arm length the median is 111·04, and the probable error 12·90, whilst of their oral arm length the median is 103·83, and the probable error 10·99. The results are shown in the table. In the one half the larvæ have been sorted into groups in which the aboral arm lengths are constant, and the mean value of the oral arm length has been determined in each group, and in the other, into groups in which the oral arm lengths are constant.

Length of AC.	Mean associated length of AD.	$\frac{AC-M_{AC}}{Q_{AC}}$	$\frac{AD-M_{AD}}{Q_{AD}}$	Length of AD.	Mean associated length of AC.	$\frac{AD-M_{AD}}{Q_{AD}}$	$\frac{AC-M_{AC}}{Q_{AC}}$
60 to 64·9	72·60	−3·76	−2·89				
65 „ 69·9	83·94	−3·38	−1·86	65 to 69·9	73·77	−3·31	−2·94
70 „ 74·9	75·51	−2·99	−2·62	70 „ 74·9	77·98	−2·85	−2·60
75 „ 79·9	74·02	−2·60	−2·76	75 „ 79·9	81·47	−2·40	−2·33
80 „ 84·9	86·04	−2·21	−1·66	80 „ 84·9	94·33	−1·94	−1·33
85 „ 89·9	84·52	−1·82	−1·80	85 „ 89·9	91·47	−1·49	−1·55
90 „ 94·9	92·88	−1·44	−1·04	90 „ 94·9	102·05	−1·03	− ·74
95 „ 99·9	96·96	−1·05	− ·67	95 „ 99·9	105·06	− ·58	− ·50
100 „ 104·9	99·91	− ·66	− ·40	100 „ 104·9	109·35	− ·12	− ·17
105 „ 109·9	100·36	− ·27	− ·36	105 „ 109·9	117·13	+ ·33	+ ·43
110 „ 114·9	106·89	+ ·11	+ ·23	110 „ 114·9	116·89	+ ·79	+ ·42
115 „ 119·9	109·96	+ ·50	+ ·48	115 „ 119·9	122·47	+1·24	+ ·85
120 „ 124·9	115·31	+ ·89	+1·00	120 „ 124·9	129·79	+1·70	+1·41
125 „ 129·9	116·04	+1·28	+1·16	125 „ 129·9	129·51	+2·15	+1·39
130 „ 134·9	120·69	+1·66	+1·49	130 „ 134·9	137·13	+2·61	+1·98
135 „ 139·9	121·32	+2·05	+1·55	135 „ 139·9	149·53	+3·06	+2·95
140 „ 144·9	126·83	+2·44	+2·05	140 „ 144·9	138·33	+3·52	+2·08
145 „ 149·9	140·85	+2·83	+3·32	145 and over	147·32	+3·97	+2·77
150 „ 154·9	131·45	+3·21	+2·47				
155 and over	141·51	+3·60	+3·38				

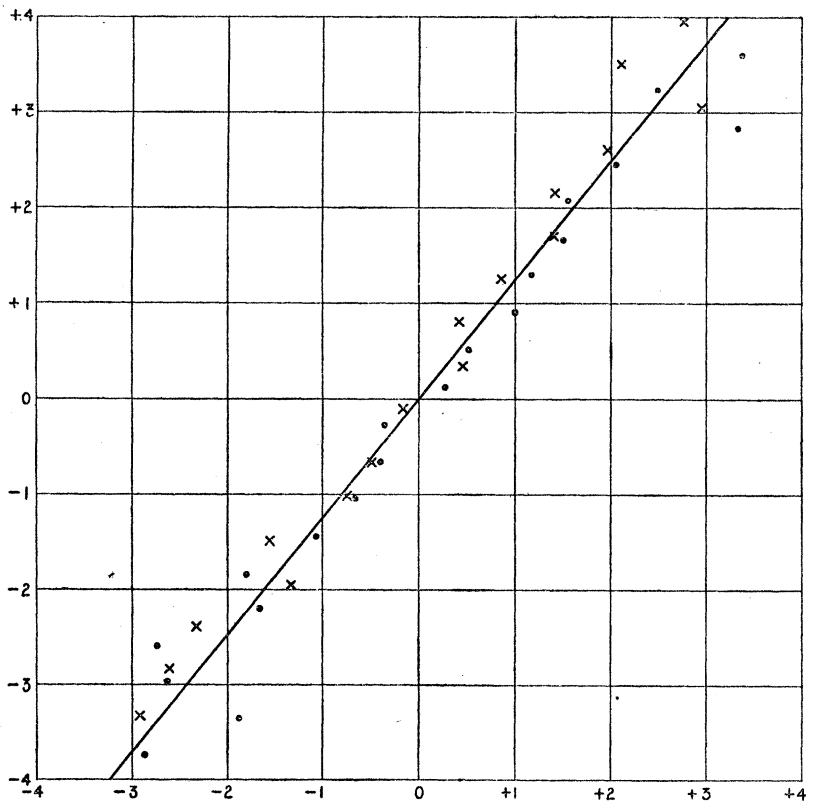
The mean value of r, which agrees best with the various values in these two sets of observations, is ·81. In Fig. 10, all the determinations of r are indicated. In each

* 'Roy. Soc. Proc.,' vol. 45, p. 135.

† 'Roy. Soc. Proc.,' vol. 51, p. 2.

case the deviations of the arm length whose value is fixed is measured on the ordinates, and the mean deviations of the associated arm length on the abscissæ. The values obtained in the first half of the table are indicated by dots, and those in the second half by crosses. The diagonal straight line indicates the ratio ·81. All but the extreme values agree fairly well, considering the comparatively small number of measurements subjected to examination. We may conclude, therefore, that the variations in the size of the aboral and oral arm lengths in this particular group of larvæ have a practically constant relation to one another. We have already seen

Fig. 10.



that this relation is not absolutely constant, but is affected by changes in the environmental conditions such as temperature, and hence it was thought useless to subject any more of the measurements to this test of correlation. In five local races of shrimps, WELDON* found the relation of the carapace length to post-spinous length to vary from ·80 to ·85. GALTON had previously suggested that a relation such as this would probably be constant in all races of the same species, and WELDON seems inclined to agree with this view, and to consider that these deviations may be due to incidental error. This conclusion seems to me to be scarcely justified by facts. Thus, if a rise from 17° to 23° in the temperature of the water during development causes

* 'Roy. Soc. Proc.,' vol. 51, p. 2.

an increase of 4·1 per cent. in the ratio of the arm lengths of plutei, the differences of temperature, salinity of the sea-water, and other conditions, under which shrimps obtained from the various localities had developed, would be sufficient to account for the differences found by WELDON in the values of r, without looking elsewhere for an explanation.

The above comprise all the results obtained by statistical examination. As the measurements were not very suitable for the purpose, it was not thought worth while to deal with them at any greater length. Such as they are, they serve to prove that Echinoderm larvæ vary from the average in respect of the size of their various parts according to the law of frequency error, in the same manner as GALTON found to be the case for man, and WELDON for shrimps and crabs. Their most obvious divergence from the results of these authors lies in their much greater variability. The probable error of the body length is 6·1 per cent., and of the aboral and oral arm lengths respectively 11·4 per cent. and 9·5 per cent. GALTON* found the probable error of the stature in man to be 2·6 per cent., and of the height of the right knee, which showed the greatest amount of variation of the parts measured, to be 3·9 per cent. WELDON found the probable error of the carapace length in shrimps obtained from Plymouth to be 1·81 per cent. and in shrimps obtained from Sheerness 1·23 per cent. The telson length, which showed the greatest amount of variation, had a probable error of 2·4 per cent. Lastly, the carapace length of Naples specimens of *Carcinus mœnas* had a probable error of 1·07 per cent., whilst the proximal portion of the right chela, which showed the greatest amount of variation, had a probable error of 5·8 per cent. Thus, with the exception of this last measurement, the body length of the larvæ has almost twice as great a probable error as any of the organs measured by GALTON and WELDON, whilst those of the arm lengths are three or four times as great.

General Conclusions.

It may not be out of place to make a few general remarks as to the bearing of the conclusions arrived at upon the problems of variation and natural selection. We must conclude that the larvæ show a considerable amount of variation in their size, quite apart from any influences caused by conditions of environment. These variations WEISMANN considers to be due to actual differences of the germ plasm. What I have sought to establish in this paper is that this variation may be considerably increased by the operation of changes in the environment during development. We have seen that the body length of the larvæ has, on an average, a probable error of 6·1 per cent. We have also seen that larvæ allowed to develop in water diluted by the addition of 50 cub. centims. of distilled water per litre are increased in size by some 15 per cent. If, therefore, half of a number of developing larvæ were subjected to this changed

* 'Roy. Soc. Proc.,' vol 44, 137.

environmental condition, whilst the other half remained under the old conditions, the variability of the whole number of larvæ would be more than trebled. In the same way other conditions, such as a high or low temperature, might operate to produce a decrease in the size of the larvæ. Now, other things being equal, the greater the variation in any group of organisms, the greater chance have these organisms as a whole of becoming modified by the action of natural selection, and hence, as probably no organisms are as perfectly adapted to their surroundings as it is possible for them to be, it is of importance to them that they should be as variable as possible, so as by the action of natural selection to become still better adapted. Perhaps one of the most important points established in this paper is that the ovum is very much more sensitive to the conditions of its environment, such as temperature, at the time of impregnation, than subsequently. Thus, whilst the temperature of the water in which these larvæ develop under natural conditions may show but slight variations on an average, in periods extending over several weeks, yet the extreme variations within such a period may be considerable. As, therefore, ova would probably happen to undergo impregnation at all times in the breeding season, whatever the temperature of the water, the larvæ developed therefrom would have a greater variability than if the ova had been no more sensitive to temperature at one time than at another. Though it has not been conclusively proved that the ovum is specially sensitive at the time of impregnation to other conditions than that of temperature, yet the probability is that this is the case, and that conditions such as the salinity of the water also have a more powerful influence. There is a condition which has been left untouched in the present paper, which probably is of considerable importance in nature. It is that of the freshness of the ova and spermatozoa at the time of impregnation. Probably the ova frequently remain unfertilised for some time after they have been ejected from the ovaries, and they may be frequently fertilised by stale spermatozoa. In such cases we can scarcely doubt that the larvæ so produced will vary from those developed from ova fertilised, immediately after ejection, by fresh spermatozoa.

Another conclusion arrived at, which is of some importance in the study of the problems of variation, is that changes of environment may produce different and opposite effects upon different parts of the same organism. Thus, a fall in the temperature or a decrease in the salinity of the water produces a decrease in the arm length of the larvæ, and an increase in the body length. It is thus possible for parts of an organism to become modified, though they may be entirely unoperated upon by the action of natural selection, or may even serve some useful purpose to the animal. If, for instance, it is of greater utility to the larvæ that their body lengths should increase than that their arm lengths should increase, and if by the operation of a fall in the temperature of the water larvæ with greater body lengths and smaller arm lengths are produced, then, on an average, the larvæ exhibiting these changed characteristics to the most marked extent will survive, and the race will be modified in this direction.

Summary.

The following are the chief conclusions arrived at in this paper:—

If the ova of *Strongylocentrotus lividus* be placed in water at about 8° or 25° C. for an hour or even for a minute at the time of impregnation, the resulting plutei, after eight days development, are some 4·4 per cent. smaller than those of ova impregnated at from 17° to 22°. If kept at the abnormal temperature for only ten seconds during impregnation, the resulting larvæ are only 1·7 per cent. smaller, probably because the time is too short for all the ova to become impregnated under the abnormal conditions.

Larvæ allowed to develop in water at about 17° to 22° are 2 per cent. or more larger than those allowed to develop at temperatures above or below these limits.

Larvæ obtained from artificial fertilisations made in the middle of August are about 20 per cent. smaller than those obtained in April, May, and October, whilst those obtained in June and July are intermediate in size. This is probably due to the comparative immaturity of the ova and spermatozoa in the off-breeding season.

Larvæ allowed to develop in water containing 50 cub. centims. of distilled water per litre are 15·6 per cent. larger than those grown under normal conditions, and those in water containing 25 cub. centims. per litre 9·5 per cent. larger. On the other hand, in water containing 150 cub. centims. distilled water per litre they are 4·3 per cent. smaller. Larvæ developed in water more concentrated than the normal remain practically unchanged, but those grown under normal conditions from ova impregnated in concentrated water are 1·6 per cent. larger.

Larvæ grown in semi-darkness are 2·5 per cent. larger than those grown under normal conditions, whilst those grown in absolute darkness are 1·3 per cent. smaller. Those grown in the blue light of copper sulphate solution are 4·5 per cent. smaller, and in the violet-blue light of Lyons blue solution 7·4 per cent. smaller. Those grown in the green light of nickel nitrate solution are 4·8 per cent. smaller, and those in red and yellow lights respectively 6·9 per cent. and 8·9 per cent. smaller.

The body length of the larvæ is uninfluenced by the number of larvæ developing together in a given volume of water, if it be kept below 30,000 per litre. It is also only slightly influenced by the amount of sperma added on impregnation.

Larvæ grown in water containing 1 in 70,400 of uric acid are 12·2 per cent. *larger* than those grown under normal conditions, and only when the proportion of uric acid is increased to 1 in 28,000 is an unfavourable influence exerted, the larvæ being in this case 2·1 per cent. smaller than the normal. In water containing about 1 in 60,000 of urea the larvæ are about 3 per cent. larger. Larvæ grown in water in which other larvæ have previously developed are 7·0 per cent. smaller than the normal.

Larvæ grown in water containing an additional amount of carbonic acid gas are slightly larger than the normal. Even if the amount of carbonic acid be only just insufficient to kill the larvæ, an unfavourable effect is not exerted on the growth.

The larvæ are not much influenced by partial deaëration or by oxygenation of the water in which they are developing.

The aboral and oral arms of the larvæ reach their maximum growth after eight days' development, after which they begin to undergo absorption. The body length increases regularly up to the sixteenth day, this being as far as it was examined.

On an average, the aboral and oral arms of larvæ grown in water containing 4000 larvæ per litre are respectively 13·4 and 15·9 per cent. shorter than of those in water containing 500 per litre; in water containing 17,500 per litre they are 25·9 and 23·3 per cent. shorter, and in water containing over 30,000 per litre, 53·0 and 43·2 per cent. shorter.

The arms of larvæ developed from ova impregnated at 8° C. are about 8 per. cent. shorter than the normal, and those at 25° C. about 2·5 per cent. shorter. In larvæ developed in water at temperatures above 22°, the aboral and oral arms are respectively 10·8 per cent. and 8·5 per cent. *longer* than those developed at 18° to 20°. The ratio between the arm lengths is 4·3 per cent. higher at temperatures above 22° than at those below 18°.

The body length of larvæ developed in diluted water is on an average increased by 9·1 per cent., whilst the arm lengths are decreased by 7·7 per cent. and 10·5 per cent.; hence, as the arm lengths are percentages on the body lengths, the absolute arm lengths are not affected at all.

The arm lengths of larvæ developed in semi-darkness, absolute darkness, green and violet blue lights are 10 per cent. or more shorter than of those grown under normal conditions.

Statistical examination of the measurements of the body length of the larvæ showed that the deviations from the average occur with a frequency indicated by the theoretical law of error. Larvæ with body lengths above 32·66 have a relative probable error of about ·057, those with smaller body lengths than this one of about ·063.

The variability of the larvæ, in respect of the body length, steadily declines as the period of development proceeds from the fifth day. The variability reaches a maximum at 18° to 20°, the temperature most favourable for development.

The coefficients of the aboral arm measurements do not agree very well with the theoretical values, possibly owing to dimorphism, but those for the oral arms are in good agreement. In the one case in which they were examined the arm lengths appeared to be correlated to each other, in fairly close agreement with the theoretical relation.

The probable error of the body length is 6·1 per cent., of the aboral arm length 11·3 per cent., and of the oral arm length 9·4 per cent., this being a much greater variability than has been found in other animals.

In conclusion, I wish to offer my warmest thanks to several of the Staff of the Zoological Station at Naples for the great kindness and ready assistance they have invariably shown me during my occupation of the Oxford University table, and also to Professor BURDON SANDERSON for reading over the manuscript of this paper.

Number of experiment.	Date of fertilisation.	Echini used for fertilisation.	Temperature of impregnation.	Mean temperature during development.	Time of development in days.	Number of larvæ per litre.	Conditions of development.	Mean body length.	Mean aboral arm length.	Mean oral arm length.	Corrected aboral arm length.	Corrected oral arm length.	Ratio of arm lengths.	Variability.
1	April 4	2 ♀ 1 ♂	13·5	16·7	5	..	Kept 1 hour at 13°·5 during impregnation	32·11	60·70	66·26	..	..	·91	26
2	,,	,,	,,	,,	5	..	,, ,, ,,	32·08	60·02	60·86	..	..	·99	18
3	,, 6	3 ♀ 1 ♂	7·6	16·3	4	..	,, 7°·6 ,,	29·48	52·66	58·45	..	..	·90	33
4	,,	,,	,,	,,	5	..	,, ,, ,,	29·87	52·96	60·54	..	..	·87	22
5	,, 12	1 ♀ 2 ♂	17·7	17·6	3	..	Normal ,, ,,	30·56	60·00	63·42	..	..	·94	24
6	,, 13	3 ♀ 3 ♂	21·3	17·8	4	..	,, ,, ,,	31·55	69·76	80·05	..	..	·87	19
7	,,	,, ,,	23·8	,,	5	..	Kept 1 hour at 23°·8 during impregnation	31·85	72·46	84·07	..	..	·86	25
8	,,	,, ,,	,,	,,	4	..	,, ,, ,,	30·77	66·92	74·72	..	..	·90	23
9	,,	,, ,,	,,	,,	5	..	,, ,, ,,	31·44	68·08	78·07	..	..	·87	31
10	,, 14	2 ♀ 3 ♂	17·5	18·0	5	..	Normal ,, ,,	33·30	71·16	84·35	..	..	·84	15
11	,,	,, ,,	,,	,,	4	..	,, ,, ,,	32·33	72·82	84·79	..	..	·86	21
12	,,	,, ,,	,,	,,	6	..	,, ,, ,,	33·43	78·76	91·85	..	..	·86	18
13	,, 17	2 ♀ 1 ♂	7·9	17·1	5	..	Kept 1 hour at 7°·9 during impregnation	28·82	65·65	72·23	..	..	·91	38
14	,,	,,	,,	,,	6	..	,, ,, ,,	29·44	84·85	84·99	..	..	1·00	27
15	,,	,, ,,	,,	,,	7	..	,, ,, ,,	30·01	89·22	87·04	..	..	1·03	29
16	,,	,, ,,	10·4	,,	4	..	,, 10°·4 ,,	30·30	59·62	63·33	..	..	·94	30
17	,,	,, ,,	,,	,,	5	..	,, ,, ,,	30·38	60·63	64·17	..	..	·94	31
18	,,	,, ,,	,,	,,	6	..	,, ,, ,,	31·99	75·35	75·38	..	..	1·00	29
19	,,	,, ,,	13·5	,,	6	..	,, 13°·5 ,,	29·90	60·28	64·64	..	..	·93	33
20	,, 24	2 ♀ 1 ♂	19·8	16·0	6	2,400	Normal ,, ,,	33·39	102·29	103·13	..	..	·99	19
21	,,	,, ,,	,,	,,	8	,,	,, ,, ,,	34·95	111·24	107·72	116·6	112·9	1·03	19
22	,,	,, ,,	,,	,,	10	,,	,, ,, ,,	34·98	112·69	107·01	..	..	1·05	18
23	,,	,, ,,	25·1	,,	6	9,500	Kept 1 hour at 25°·1 during impregnation	33·63	94·53	97·55	..	..	·97	12
24	,,	,, ,,	,,	,,	8	,,	,, ,, ,,	33·89	106·43	104·65	126·6	124·5	1·02	13
25	,, 25	3 ♀ 3 ♂	15·5	16·0	6	2,660	Normal ,, ,,	33·18	103·81	99·22	..	..	1·05	22
26	,,	,, ,,	,,	,,	8	,,	,, ,, ,,	33·24	110·82	101·87	116·7	107·3	1·09	22
27	,,	,, ,,	,,	,,	10	,,	,, ,, ,,	33·33	106·94	100·19	..	..	1·07	22
28	,,	,, ,,	,,	,,	6	7,400	,, ,, ,,	32·65	96·10	99·71	..	..	·96	26
29	,,	,, ,,	,,	,,	8	,,	,, ,, ,,	34·39	86·37	92·60	99·2	106·4	·93	18
30	,,	,, ,,	,,	,,	10	,,	,, ,, ,,	34·17	78·52	90·04	..	..	·87	21
31	,,	,, ,,	,,	16·2	14	,,	,, ,, ,,	35·35	79·58	81·87	..	..	·97	34
32	,,	,, ,,	,,	16·0	6	18,650	,, ,, ,,	32·86	77·03	81·78	..	..	·94	20
33	,,	,, ,,	,,	,,	8	,,	,, ,, ,,	33·53	74·99	85·64	108·0	117·6	·88	22
34	,,	,, ,,	,,	16·2	14	,,	,, ,, ,,	34·62	68·26	73·80	..	..	·92	18
35	,,	,, ,,	,,	16·0	8	27,600	,, ,, ,,	34·10	58·65	72·21	91·0	112·1	·81	26
36	,,	,, ,,	,,	,,	8	39,000	,, ,, ,,	31·64	42·25	61·70	75·2	109·8	·68	32
37	,,	,, ,,	,,	,,	8	600	Developed in water containing 20 times as much sperma as other fertilisations	30·53	68·20	78·54	69·0	79·4	·87	19
38	May 9	3 ♀ 3 ♂	21·6	17·2	6	1,740	Normal ,, ,,	34·55	113·43	109·41	..	..	1·04	28
39	,,	,, ,,	,,	,,	8	,,	,, ,, ,,	36·08	126·19	116·26	130·6	120·3	1·07	25
40	,,	,, ,,	16·4	,,	6	540	,, ,, ,,	32·66	84·63	90·95	..	..	·93	27
41	,,	,, ,,	,,	,,	8	105	,, ,, ,,	35·75	130·37	122·97	130·6	123·2	1·06	25
42	,,	,, ,,	,,	,,	8	310	,, ,, ,,	34·12	118·73	114·25	119·5	115·0	1·04	26

Number of experiment.	Date of fertilisation.	Echini used for fertilisation.	Temperature of impregnation.	Mean temperature during development.	Time of development in days.	Number of larvæ per litre.	Conditions of development.	Mean body length.	Mean aboral arm length.	Mean oral arm length.	Corrected aboral arm length.	Corrected oral arm length.	Ratio of arm lengths.	Variability.
43	May 9	3 ♀ 3 ♂	7·5	17·2	8	3,240	Kept 1 hour at 7°·5 during impregnation	33·01	121·66	116·78	129·7	124·5	1·04	23
44	,,	,, ,,	11·5	,,	8	2,840	,, 11°·5 ,,	35·69	122·65	114·73	129·0	120·2	1·06	27
45	,, 12	3 ♀ 3 ♂	24·9	17·8	8	7,900	Kept 5 minutes at 24°·9 ,,	33·54	119·81	107·22	138·8	124·2	1·12	22
46	,,	,, ,,	12·5	,,	8	10,900	,, 12°·5 ,,	34·65	110·58	107·37	134·9	131·0	1·03	21
47	,,	,, ,,	6·9	,,	8	8,440	,, 6°·9 ,,	32·73	112·75	105·05	131·5	122·8	1·07	17
48	,, 17	4 ♀ 3 ♂	17·6	19·3	8	2,700	Kept at 23° for the ten hours subsequent to impregnation. No. 49 kept 1 hour at 28°·1 during impregnation	35·53	118·65	112·20	125·1	118·3	1·06	35
49	,,	,, ,,	23·1	,,	8	5,200		34·29	89·69	91·81	99·0	103·0	·98	24
50	,,	,, ,,	17·6	,,	8	6,800	Kept at 12° for the ten hours subsequent to impregnation. No. 51 kept 1 hour at 11°·2 during impregnation	34·68	108·60	108·83	123·4	123·7	1·00	32
51	,, 21	,, ,,	11·2	,,	8	2,140		31·79	77·06	95·77	80·3	99·9	·80	32
52	,,	3 ♀ 3 ♂	19·3	19·2	8	4,120	Normal	33·95	111·54	112·56	124·5	121·8	1·02	20
53	,,	,, ,,	22·1	,,	8	2,020	Kept at about 22°·1 during the whole period of development. No. 54 kept 1 hour at 22°·9 during impregnation	33·38	127·05	114·99	132·2	119·6	1·10	20
54	,,	,, ,,	22·9	,,	8	1,100		34·43	149·50	132·82	152·8	135·7	1·13	26
55	,,	,, ,,	19·3	15·7	8	1,960	Kept at about 15°·7 during the whole period of development. No. 56 kept 1 hour at 15°·0 during impregnation	32·45	94·93	98·45	97·7	101·3	·96	25
56	,,	,, ,,	15·0	,,	8	1,900		31·61	112·60	111·39	116·9	115·6	1·01	20
57	,,	,, ,,	19·3	19·2	8	1,680	Kept in water from a previous fertilisation during development	31·27	93·66	102·09	96·8	105·5	·92	28
58	,, 25	3 ♀ 4 ♂	20·0	18·2	8	4,400	Normal	33·78	105·64	105·39	114·9	114·7	1·00	22
59	,,	,, ,,	,,	,,	8	8,250	Ten times less spermatozoa added than in normal fertilisation	33·57	68·18	77·64	79·4	90·4	·88	22
60	,,	,, ,,	,,	,,	8	580	more	29·47	89·21	94·50	90·6	96·0	·94	26
61	,,	,, ,,	25·1	,,	6	15,600	Kept 1 hour at 25°·1 during impregnation ,, ,,	29·13	77·53	91·26	..	..	·85	34
62	,,	,, ,,	,,	,,	8	,,	,, ,,	30·19	84·13	93·82	110·4	123·1	·90	33
63	,,	,, ,,	,,	,,	8	2,100	,, 3 minutes at 25°·1 ,,	31·19	108·29	106·71	112·8	111·2	1·01	29
64	,,	,, ,,	8·9	,,	8	8,650	,, 1 hour at 8°·9 ,,	33·77	84·32	89·52	98·9	105·0	·94	24
65	,,	,, ,,	,,	,,	8	8,400	,, 3 minutes at 8°·9 ,,	33·69	92·29	97·67	107·8	114·1	·95	33
66	,,	,, ,,	20·0	,,	8	3,400	Sea-urchins kept 1 hour at 5° C. previous to fertilisation	35·23	110·72	113·39	118·2	121·0	·98	33
67	,, 29	4 ♀ 3 ♂	18·6	18·7	8	15,550	Normal	33·34	77·46	85·06	101·6	111·5	·91	25
68	,,	,, ,,	,,	,,	6	26,300	Developed in absolute darkness	31·74	57·69	68·82	..	..	·83	30
69	,,	,, ,,	,,	,,	8	,,	,, ,,	32·97	61·38	75·93	93·7	115·9	·81	24
70	,,	,, ,,	,,	,,	8	17,900	,, red light	31·42	72·28	84·45	98·2	114·7	·86	26
71	,,	,, ,,	,,	,,	8	15,900	,, yellow light	31·63	59·53	76·72	78·5	100·1	·78	22
72	,,	,, ,,	,,	,,	8	18,950	,, blue light (copper sulphate)	32·02	74·74	88·00	102·1	121·4	·85	23
73	,,	,, ,,	,,	,,	8	12,400	,, semi-darkness	34·79	59·89	73·73	74·7	92·0	·81	21
74	,,	,, ,,	,,	,,	8	7,900	Kept 1 hour in absolute darkness during impregnation	32·98	112·61	101·24	130·4	117·2	1·11	26
75	June 4	3 ♀ 3 ♂	19·0	19·5	8	14,200	Normal	29·91	113·39	102·80	145·6	132·0	1·10	26
76	,,	,, ,,	,,	,,	8	6,300	Kept during development in water from a previous fertilisation	27·73	75·46	91·52	85·0	103·0	·82	35
77	,,	,, ,,	,,	,,	8	10,900	Kept in previous fertilisation water for 9 hours after impregnation	29·90	91·19	86·89	111·1	105·8	1·05	31
78	,,	,, ,,	,,	,,	8	4,300	Kept in previous fertilisation water after first 9 hours	29·39	89·91	88·64	97·6	95·2	1·01	27
79	,,	,, ,,	,,	,,	8	15,900	Developed in water containing 1 : 154,000 uric acid	31·54	112·78	105·69	148·6	139·3	1·07	33
80	,,	,, ,,	,,	,,	8	3,400	,, ,, 1 : 70,400 ,,	33·55	116·71	104·04	124·6	110·1	1·12	32
81	,,	,, ,,	,,	,,	8	13,300	,, ,, 1 : 44,000 urea	30·56	110·03	102·85	130·3	130·2	1·07	24
82	,,	,, ,,	,,	,,	8	14,000	Water stirred during development	30·02	110·23	102·54	141·1	131·2	1·07	29

Number of experiment.	Date of fertilisation.	Echini used for fertilisation.	Temperature of impregnation.	Mean temperature during development.	Time of development in days.	Number of larvæ per litre.	Conditions of development.	Mean body length.	Mean aboral arm length.	Mean oral arm length.	Corrected aboral arm length.	Corrected oral arm length.	Ratio of arm lengths.	Variability.
			°	°										
83	June 4	3♀ 3♂	19·0	19·5	8	14,500	Water not stirred at all during development	28·96	107·89	101·59	139·2	131·1	1·06	27
84	,,	,, ,,	19·2	,,	8	8,900	Kept 1 hour in previous fertilisation water during impregnation	31·18	108·12	98·01	127·4	115·5	1·10	35
85	,,	,, ,,	19·0	,,	8	3,700	Kept 1 hour in 1 : 70,400 uric acid solution during impregnation	32·17	115·05	112·58	123·6	120·9	1·02	30
86	,, 8	4♀ 1♂	20·0	19·2	6	5,400	Normal	32·99	100·44	95·75	..	..	1·06	21
87	,,	,, ,,	,,	19·1	8	,,	,,	32·93	101·04	94·60	111·9	104·8	1·07	22
88	,,	,, ,,	,,	19·0	10	,,	,,	33·45	95·75	90·45	..	..	1·06	22
89	,,	,, ,,	,,	19·2	16	,,	,,	33·91	76·46	74·77	..	..	1·02	21
90	,,	,, ,,	,,	19·1	8	4,800	Four times more spermatozoa added than in normal fertilisation	34·91	114·20	108·46	125·2	118·9	1·05	29
91	,,	,, ,,	,,	,,	8	1,750	Developed in absolute darkness	33·26	88·27	93·75	91·4	97·0	·90	18
92	,,	,, ,,	,,	,,	8	1,200	,, 850 cub. centims. of sea-water diluted to a litre	30·63	124·62	118·01	127·6	120·8	1·06	30
93	,,	,, ,,	,,	,,	8	700	Developed in 1090 cub. centims. concentrated to a litre.	32·33	112·94	99·46	114·5	100·9	1·13	32
94	,, 13	3♀ 3♂	19·2	18·4	3	2,170	Normal	28·21	76·50	77·71	..	..	·98	22
95	,,	,, ,,	,,	,,	5	,,	,,	30·58	106·38	106·91	..	..	1·00	23
96	,,	,, ,,	,,	18·5	6	,,	,,	31·20	106·49	105·01	..	..	1·01	28
97	,,	,, ,,	,,	18·7	8	,,	,,	31·15	119·85	114·28	125·0	119·2	1·05	29
98	,,	,, ,,	,,	19·0	10	,,	,,	32·43	116·71	108·68	..	..	1·07	26
99	,,	,, ,,	,,	19·5	16	,,	,,	33·17	79·90	72·92	..	..	1·09	19
100	,,	,, ,,	5·5	18·7	8	3,650	Kept 10 seconds at 5°·5 during impregnation	30·41	111·08	99·63	119·2	106·9	1·11	16
101	,,	,, ,,	,,	,,	8	1,600	,, 1 minute ,, ,, ,,	29·01	132·49	118·82	136·7	122·6	1·12	22
102	,,	,, ,,	,,	,,	8	3,600	,, 1 hour ,, ,, ,,	30·77	109·87	98·76	117·8	105·9	1·11	16
103	,,	,, ,,	9·4	,,	8	3,150	,, 10 seconds at 9°·4 ,, ,,	30·29	114·31	103·99	121·5	110·5	1·10	28
104	,,	,, ,,	,,	,,	8	1,900	,, 1 minute ,, ,, ,,	30·01	112·21	101·70	116·5	105·6	1·10	19
105	,,	,, ,,	,,	,,	8	2,300	,, 1 hour ,, ,, ,,	30·38	110·98	97·57	116·1	102·1	1·14	23
106	,,	,, ,,	25·7	,,	8	5,600	,, 10 seconds at 25°·7 ,, ,,	31·20	110·32	100·61	122·7	111·9	1·10	26
107	,,	,, ,,	,,	,,	8	5,000	,, 1 minute ,, ,, ,,	30·55	106·44	100·19	117·1	110·2	1·06	22
108	,,	,, ,,	,,	,,	8	9,700	,, 1 hour ,, ,, ,,	29·68	95·08	93·11	113·5	111·2	1·02	27
109	,,	,, ,,	19·2	,,	8	700	Water stirred during first 24 hours of development	31·08	106·61	96·91	108·1	98·3	1·10	23
110	,, 17	3♀ 3♂	19·0	19·5	8	7,200	Normal	31·95	101·13	96·02	115·7	109·8	1·05	32
111	,,	,, ,,	,,	,,	8	11,800	Normal, only developed in water from another tank	31·12	99·83	98·05	123·4	121·2	1·02	29
112	,,	,, ,,	,,	,,	8	7,500	Developed in red light	29·01	112·51	102·36	129·4	117·7	1·10	32
113	,,	,, ,,	,,	,,	8	6,200	,, yellow light	27·72	126·34	111·96	142·0	125·8	1·13	29
114	,,	,, ,,	,,	,,	8	8,100	,, blue light (copper sulphate)	30·24	102·86	97·23	119·5	113·0	1·06	29
115	,,	,, ,,	,,	,,	8	8,100	,, absolute darkness	29·58	90·83	101·46	105·5	117·9	·90	28
116	,,	,, ,,	,,	,,	8	12,500	,, green glass vessel	31·56	88·82	88·92	111·0	111·2	1·00	24
117	,,	,, ,,	,,	,,	8	20,000	Kept for 1 hour during impregnation in absolute darkness	31·38	100·60	92·05	140·8	128·9	1·09	26
118	,,	,, ,,	19·3	,,	8	12,600	Kept for 1 hour during impregnation in bright sunlight	30·60	91·49	88·58	114·5	110·9	1·03	25

Number of experiment.	Date of fertilisation.	Eshini used for fertilisation.	Temperature of impregnation.	Mean temperature during development.	Time of development in days.	Number of larvæ per litre.	Conditions of development.	Mean body length.	Mean aboral arm length.	Mean oral arm length.	Corrected aboral arm length.	Corrected oral arm length.	Ratio of arm lengths.	Variability.
			°	°										
119	June 17	3 ♀ 3 ♂	19·0	19·5	8	10,800	Developed in water containing ·0493 grm. $CaCl_2$ per litre	30·55	108·45	98·89	131·0	120·2	1·10	26
120	,, 22	4 ♀ 2 ♂	19·6	20·8	8	4,100	Normal	33·07	89·90	95·62	97·3	103·5	·94	27
121	,,	,, ,,	,,	,,	8	1,950	Developed in solution containing 1 : 28,000 uric acid . .	32·38	124·38	120·99	120·2	125·7	1·03	22
122	,,	,, ,,	,,	,,	8	5,400	,, ,, 1 : 58,000 ,, ,,	34·98	118·26	110·11	131·0	122·0	1·07	21
123	,,	,, ,,	,,	,,	8	5,100	,, ,, 1 : 76,400 ,, ,, after first day	34·73	106·72	97·69	117·6	107·7	1·09	20
124	,,	,, ,,	,,	,,	8	3,900	Developed in solution containing 1 : 65,000 urea . . .	33·85	100·29	96·83	108·1	104·4	1·04	26
125	,,	,, ,,	,,	,,	8	9,100	Water not stirred at all during development	34·07	102·74	96·29	121·4	113·8	1·07	22
126	,,	,, ,,	,,	,,	8	7,600	Developed in oxygenated water	32·11	109·05	109·56	124·5	125·1	1·00	25
127	,,	,, ,,	19·0	,,	8	10,100	Kept 1 hour during impregnation in water from previous fertilisation	33·11	88·06	87·80	105·8	105·5	1·00	25
128	,,	,, ,,	20·8	,,	8	4,300	Kept 1 hour during impregnation in water containing 1 : 58000 uric acid	31·32	92·40	87·84	100·3	95·4	1·05	15
129	,,	,, ,,	20·8	,,	8	7,000	Kept 1 hour during impregnation in oxygenated water	31·91	105·88	101·58	120·7	115·8	1·04	24
130	,, 29	4 ♀ 2 ♂	20·3	21·8	8	1,400	Normal	28·61	130·46	120·67	134·1	124·0	1·08	26
131	,,	,, ,,	,,	,,	8	1,600	Developed in 950 cub. centims. of sea-water diluted to a litre	33·06	122·03	107·69	125·9	111·1	1·13	27
132	,,	,, ,,	,,	,,	8	950	Developed in 1100 cub. centims. of sea-water concentrated to a litre	28·82	160·45	134·95	163·5	137·5	1·19	29
133	,,	,, ,,	,,	,,	8	370	Developed in deaërated water	28·13	137·97	132·93	139·0	133·9	1·04	31
134	,,	,, ,,	20·4	,,	8	4,000	Kept 1 hour during impregnation in solution containing 1 in 5 of distilled water	33·23	108·83	97·21	117·5	105·0	1·12	26
135	July 11	5 ♀ 2 ♂	20·3	23·3	8	160	Normal	29·45	112·81	97·25	113·1	97·6	1·16	31
136	,,	,, ,,	,,	,,	8	900	Developed in solution containing ·0804 grm. $CaCl_2$ per litre	29·79	111·54	93·77	113·6	95·5	1·19	24
137	,,	,, ,,	,,	,,	8	510	,, ,, ·0512 ,, ,,	29·69	98·31	85·13	99·3	86·0	1·15	21
138	,, 25	4 ♀ 2 ♂	20·3	23·7	8	1,100	Normal	27·61	118·79	108·05	121·4	110·4	1·10	21
139	,,	,, ,,	,,	19·9	8	1,350	,, ...	28·29	104·83	104·71	107·7	107·5	1·00	20
140	,,	,, ,,	,,	23·7	8	1,200	Developed in semi-darkness.	28·10	82·52	87·15	84·5	89·2	·95	30
141	,,	,, ,,	,,	,,	8	1,250	,, deaërated water	26·67	140·50	121·22	144·0	124·3	1·16	28
142	,,	,, ,,	,,	,,	8	500	,, water containing per litre 90 cub. centims. of water saturated with CO_2	28·35	129·72	110·23	131·0	111·3	1·18	29
143	,,	,, ,,	25·7	,,	8	700	Kept 1 hour at 25°·7 during impregnation.	27·88	107·21	95·83	108·7	97·2	1·12	22
144	,,	,, ,,	,,	,,	8	900	,, 1 minute ,, ,, ...	26·89	118·47	110·67	120·6	112·7	1·07	32
145	,,	,, ,,	7·5	,,	8	750	,, 1 hour at 7°·5 ,, ,, ...	25·20	100·52	100·83	102·0	102·3	1·00	31
146	,,	,, ,,	19·3	,,	8	1,900	,, 1 ,, during impregnation in water containing 250 cub. centims. CO_2 water per litre	27·03	96·86	91·58	100·5	95·1	1·06	30
147	Aug. 7	4 ♀ 1 ♂	19·7	23·1	8	3,500	Normal	27·34	132·05	114·33	141·3	122·3	1 15	23
148	,,	,, ,,	,,	,,	8	4,900	Developed in water containing 150 cub. centims. CO_2 water per litre	27·75	117·01	106·26	128·5	116·7	1·10	21
149	,,	,, ,,	,,	,,	8	6,100	Developed in water containing 75 cub. centims. CO_2 water per litre	27·74	114·54	107·38	128·5	120·5	1·07	18

Number of experiment.	Date of fertilisation.	Echini used for fertilisation.	Temperature of impregnation.	Mean temperature during development.	Time of development in days.	Number of larvæ per litre.	Conditions of development.	Mean body length.	Mean aboral arm length.	Mean oral arm length.	Corrected aboral arm length.	Corrected oral arm length.	Ratio of arm lengths.	Variability.
			°	°										
150	Aug. 7	4♀ 1♂	19·7	23·1	8	9,350	Developed in semi-darkness	27·72	123·17	115·38	146·2	137·0	1·07	19
151	,,	,, ,,	19·5	,,	8	6,100	Kept 1 hour during impregnation in bright sunlight . .	27·68	115·00	121·38	129·0	136·2	·95	23
152	,,	,, ,,	19·7	19·9	8	3,800	Normal	27·84	131·12	126·50	141·1	136·1	1·04	16
153	,,	,, ,,	,,	,,	8	2,150	Developed in 875 cub. centims. of sea-water diluted to a litre	29·21	104·95	95·51	109·5	99·6	1·10	20
154	,,	,, ,,	,,	,,	8	800	,, 975 ,, ,, ,, ,,	30·53	139·56	131·29	138·1	133·4	1·04	19
155	,,	,, ,,	,,	,,	8	2,150	,, 1150 ,, ,, concentrated ,,	27·88	127·91	107·93	133·4	112·5	1·18	30
156	,,	,, ,,	,,	,,	8	1,000	Kept 1 hour during impregnation in 875 cub. centims. of water diluted to a litre	28·43	105·05	115·41	107·2	117·7	·91	25
157	,, 16	4♀ 2♂	20·7	22·5	8	700	Normal	27·07	150·20	156·14	152 3	158·3	·97	18
158	,,	,, ,,	,,	,,	8	200	Five times as many spermatozoa as in normal fertilisation	28·45	124·55	143·77	125·0	144·3	·87	24
159	,,	,, ,,	,,	,,	8	250	Developed in water containing 1 : 59,000 urea	28·08	155·94	155·84	156·7	156·6	1·00	23
160	,,	,, ,,	,,	,,	8	650	,, blue light (Lyons blue solution)	25·40	106·68	127·78	108·1	129·4	·83	29
161	,,	,, ,,	,,	,,	8	250	,, absolute darkness	27·35	120·42	145·44	121·0	146·2	·83	25
162	,,	,, ,,	19·4	,,	8	2,350	Kept 1 hour during impregnation in 1 : 79,400 uric acid solution	27·70	136·47	140·84	142·9	147·5	·92	28
163	,,	,, ,,	20·5	,,	8	350	Kept 1 hour during impregnation in 1115 cub. centims. water concentrated to a litre	28·62	144·87	149·50	145·9	150·6	·97	28
164	,, 25	5♀ 2♂	20·3	23·3	8	2,100	Normal	30.09	129·36	137·26	134·8	143·0	·94	34
165	,,	,, ,,	,,	,,	8	1,150	Developed in water containing 175 cub. centims. CO_2 water per litre	29·75	141·76	143·56	144·0	146·9	·99	28
166	,,	,, ,,	,,	,,	8	2,650	Developed in water containing 75 cub. centims. CO_2 water per litre	30·13	121·88	121·14	128·3	127·6	1·01	26
167	,,	,, ,,	,,	,,	8	1,550	Developed in blue light (Lyons blue solution)	27·51	97·12	112·63	100·1	116·1	·86	25
168	,,	,, ,,	,,	,,	8	2,600	,, green light (nickel nitrate)	30·73	121·67	113·62	128·0	119·5	1·07	22
169	,,	,, ,,	20·0	,,	8	1,500	Kept 1 hour during impregnation in 1075 cub. centims. water concentrated to a litre	31·06	148·42	161·30	152·9	166·1	·92	27
170	,,	,, ,,	20·3	20·7	8	1,300	Normal	30·60	101·99	107·53	104·6	109·7	·95	28
171	,,	,, ,,	,,	,,	8	2,350	Developed in 850 cub. centims. water diluted to a litre .	30·12	117·76	110·32	123·3	115·5	1·07	25
172	,,	,, ,,	,,	,,	8	4,250	,, 925 ,, ,, ,, ,, .	33 72	87·04	88·37	94·4	95·9	·98	26
173	,,	,, ,,	,,	,,	8	2,200	,, 1075 ,, ,, concentrated ,, .	30·21	107·15	107·44	111·9	112·2	1·00	27
174	,,	,, ,,	20·4	,,	8	650	Kept 1 hour during impregnation in 850 cub. centims. water diluted to a litre	32·43	139·08	138·55	140·9	140·4	1·00	21
175	,,	,, ,,	20·6	,,	8	1,200	Kept 1 hour during impregnation in 1150 cub. centims. concentrated to a litre	31·50	130·77	135·95	133·9	139·2	96	23
176	Sept. 14	3♀ 2♂	20·0	22·2	8	17,500	Normal	34·99	100·93	93·19	136·3	125·8	1·08	21
177	,,	,, ,,	,,	,,	8	14,500	Five times as many spermatozoa as in normal fertilisation	33·77	110·53	99·17	142·6	127 9	1·11	18
178	,,	,, ,,	,,	,,	8	18,700	Ten times less spermatozoa than in ,, ,,	35·17	100·47	94·19	138·0	129·4	1·07	25
179	,,	,, ,,	,,	,,	8	11,900	Developed in deaërated water	35·39	100·81	92·76	124·8	114·8	1·09	21
180	,,	,, ,,	,,	,,	8	11,200	,, oxygenated ,, ...	35·51	116·27	103·97	142·3	127·3	1·12	25
181	,,	,, ,,	,,	,,	8	7,800	,, green light	32·43	95·40	92·33	110·3	106·7	1·03	32
182	,,	,, ,,	19·8	,,	8	6,800	Kept 1 hour during impregnation in 750 cub. centims. water diluted to a litre	34·36	127·10	108·30	144·4	123·0	1·18	20
183	,,	,, ,,	20·0	,,	8	20,300	Developed in 987·5 cub. centims. water diluted to a litre .	36·75	98·58	88·81	138·6	124·8	1·11	21

Number of experiment.	Date of fertilisation.	Echini used for fertilisation.	Temperature of impregnation.	Mean temperature during development.	Time of development in days.	Number of larvæ per litre.	Conditions of development.	Mean body length.	Mean aboral arm length.	Mean oral arm length.	Corrected aboral arm length.	Corrected oral arm length.	Ratio of arm lengths.	Variability.
			°	°										
184	Sept. 14	3 ♀ 2 ♂	25·7	22·2	8	15,800	Kept 1 hour at 25°·7 during impregnation	33·21	103·19	93·81	135·8	123·5	1·10	23
185	,,	,, ,,	8°·3	,,	8	7,200	,, ,, 8°·3 ,, ,, ...	34·11	109·75	97·40	125·5	111·4	1·13	26
186	,,	,, ,,	,,	,,	8	17,900	,, 1 minute ,, ,, ...	34·16	90·23	85·31	122·5	115·8	1·06	30
187	,,	,, ,,	,,	,,	8	11,000	,, 10 seconds ,, ,, ...	34·34	91·19	85·27	111·3	104·0	1·07	20
188	,, 25	3 ♀ 3 ♂	20·0	20·7	8	11,100	Normal	31·24	106·17	99·05	129·7	121·0	1·07	23
189	,,	,, ,,	,,	,,	8	7,900	Water not stirred at all during development	31·39	98·73	94·56	114·3	109·5	1·04	26
190	,,	,, ,,	,,	,,	8	5,900	,, stirred continuously ,, ...	30·32	104·68	101·26	117·0	113·2	1·03	22
191	,,	,, ,,	,,	,,	8	12,900	Developed in oxygenated water	30·51	94·40	89·15	123·6	112·2	1·06	25
192	,,	,, ,,	,,	,,	8	2,000	,, green light	28·97	98·27	115·78	101·3	120·4	·85	25
193	,,	,, ,,	16°·0	,,	8	11,200	Kept 1 hour at 16°·0 during impregnation	30·21	97·00	93·88	118·7	114·9	1·03	26
194	,,	,, ,,	19·7	,,	8	22,100	,, ,, during impregnation in 950 cub. centims. water diluted to a litre	31·10	98·03	95·15	141·4	137·2	1·03	24
195	,,	,, ,,	20·4	,,	8	18,700	Kept 1 hour during impregnation in 1250 cub. centims. water concentrated to a litre	30·37	112·67	105·95	154·8	145·6	1·06	27
196	,,	,, ,,	19·8	,,	8	22,200	Kept 1 hour during impregnation in 1100 cub. centims. water concentrated to a litre	30·17	95·97	93·52	138·6	135·0	1·03	22
197	,,	,, ,,	19·4	,,	8	19,100	Kept 1 hour during impregnation in water containing 1 : 61,600 uric acid	30·47	98·77	96·33	136·5	133·1	1·03	26
198	Oct. 8	3 ♀ 3 ♂	19·5	17·8	8	12,500	Normal	33·79	105·96	102·08	132·4	127·6	1·04	21
199	,,	,, ,,	23·8	,,	8	8,400	Kept 1 hour at 23°·8 during impregnation	33·66	114·81	108·95	134·1	127·3	1·05	21
200	,,	,, ,,	19·8	,,	8	31,300	,, ,, during impregnation in 1150 cub. centims. water concentrated to a litre	34·41	71·01	75·26	115·4	122·3	·94	19